Mechanisms of Maintenance and Restoration of Plant Diversity

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I dedicate this thesis to my grandfather Fernand Hautier

"We abuse land because we regard it as a commodity belonging to us.

When we see land as a community to which we belong,

we may begin to use it with love and respect."

(Aldo Leopold)

Contents

Summary

Human activities are triggering some of the most rapid losses of biodiversity in the history of life on Earth. Eutrophication, overexploitation, habitat destruction and fragmentation are the main drivers of this decrease in species richness. There is increasing evidence that this reduction of diversity will have dramatic impacts on the functioning of the natural ecosystems of the world, and on their ability to provide society with a variety of essential ecosystem services. It is therefore urgent to understand the causal mechanisms responsible for the maintenance of diversity and their potential use for restoration to develop effective conservation policies. In this thesis, I examine several potential mechanisms for the maintenance or loss of plant diversity. These include resource competition (competition for light and nutrients) and the effects of natural enemies (plant hemiparasites and seed predators).

In **Chapter 1**, I use measurements of light interception in grass monocultures to predict competitive outcomes in pairwise mixtures under conditions of varying productivity and disturbance. My findings confirm earlier success in predicting short-term competitive outcomes under productive conditions. Competition for light is asymmetric in relation to differences in light intercepting ability. In other words dominant species with an initial advantage in light intercepting ability benefit disproportionately and displace subordinate species. However, under unproductive and disturbed conditions the initial advantage of dominant species is not leading to disproportionate competitive ability. My results suggest that the asymmetry in competition for light is a consequence of eutrophication producing strong size inequality within a population that has important effects on the outcome of interspecific competition and on ecosystem properties.

In **Chapter 2**, I test whether the reduction of diversity following fertilization is due to an increase in aboveground competition for light or to an increase in both above and belowground competition ("Grime-Tilman" debate). I use a fully-factorial combination of nutrient addition and supplementary understory illumination (both either applied or not applied) for two years to show that addition of light to the grassland understory maintains plant diversity despite the additional nutrient inputs. I also show that competition for soil resources plays no detectable role in diversity loss. My results explain the particular threat of eutrophication to plant diversity by revealing increased asymmetric competition for light as a major underlying mechanism and emphasize the need to control nutrient enrichment if plant diversity is to be preserved (Hautier *et al.*, 2009).

In **Chapter 3**, I present and test a simple model of the host-parasite interaction in which parasite growth rate is a function of host growth rate. The model predicts that the combined mass of the host-parasite system is always less than the mass of the host grown alone. The model also predicts that the parasite should adopt an intermediate virulence to maximise its own performance, but that the optimum virulence depends on

host growth characteristics. To test the key assumption of the model I assess the performance of *Rhinanthus alectorolophus*, a widespread hemiparasitic annual plant, on nine common European grass species. I demonstrate that *Rhinanthus* performance is strongly correlated with the growth rate of the host grass species, thus validating the key assumption of the model, but that grasses may differ in their resistance to parasitism. Parameterising such a model of the host-parasite relationship could help to explain variation in *Rhinanthus* performance on different hosts; variation in the effects of hemiparasites in grasslands of different productivity; and differences in virulence among parasite populations.

In **Chapter 4**, I report a short-term experiment assessing the effect of seed predator body size on the recruitment of tropical forest tree seedlings in Bornean logged forest. I manipulate seed density in plots beneath con- and heterospecific adult trees and excluded predators of different body sizes using cages. I show that when larger predators were excluded small-bodied predators reduced survival of seeds from the mother tree generating an advantage for recruitment of seed from other species (the critical process of the Janzen-Connell hypothesis). This effect is cancelled out when larger predators have access to the seeds. My results show that small predator choice to select specific seeds is crucial in primary forest, logged forest or forest fragments when larger predators are lacking.

Zusammenfassung

Menschliche Aktivitäten lösen den schnellsten Verlust an Arten aus seit es Leben auf der Erde gibt. Überdüngung, Raubbau, Habitatzerstörung und Fragmentierung sind die Hauptursachen für die Abnahme des Artenreichtums. Hinweise erhärten sich, dass diese Abnahme an Biodiversität dramatischen Einfluss haben wird auf die Funktion der natürlichen Ökosysteme weltweit und deren Fähigkeit die Gesellschaft weiterhin mit vielfältigen und essentiellen Dienstleistungen zu versorgen. Darum ist es dringend nötig die kausalen Mechanismen für den Erhalt der Vielfalt und deren möglichen Nutzen für die Wiederherstellung zu verstehen, um effektive Umweltschutz Richtlinien zu entwickeln. In dieser Dissertation untersuche ich mehrere mögliche Mechansimen für den Erhalt oder den Verlust der Pflanzenvielfalt. Diese schliessen Konkurrenz um Ressourcen (Konkurrenz um Licht und Nährstoffe) und Effekte von natürlichen Feinden (pflanzliche Halbparasiten und Samenfresser) mit ein.

Im **Kapitel 1**, benutze ich Messungen der Lichtaufnahme in Grasmonokulturen um die Wirkung der Konkurrenz bei paarigen Mischungen vorauszusagen, die unterschiedlichen Graden an Produktivität und Störungen ausgesetzt werden. Meine Ergebnisse unterstützen frühere kurzzeitige Studien, die die Wirkung der Konkurrenz bei Düngung voraussagen. Konkurrenz um Licht ist asymmetrisch in Bezug auf Unterschiede in der Fähigkeit Licht aufzunehmen. In anderen Worten, dominante Arten, welche anfänglich einen Vorteil in Bezug auf die Lichtaufnahme haben, profitieren in der Folge überproportional und verdrängen schwächere Arten. Allerdings führt der anfängliche Vorteil der dominanten Arten bei ungedüngten und gestörten Bedingungen nicht zu einer überproportionalen Konkurrenzfähigkeit. Meine Resultate zeigen, dass die Asymmetrie in der Konkurrenz um Licht eine Folge des übermässigen Nährstoffeintrags ist und starke Grössenunterschiede innerhalb einer Population verursacht, was den Ausgang der interspezifischen Konkurrenz und die Eigenschaften eines Ökosystems beinflusst.

In **Kapitel 2**, teste ich ob der Artenverlust aufgrund der Düngung durch die Zunahme der Konkurrenz um Licht, also oberhalb des Bodens, ausgelöst wird oder durch eine Zunahme der Konkurrenz oberhalb und unterhalb des Bodens, also um Licht und Nährstoffe (“Grime-Tilman” Debatte). Ich verwende eine vollständig faktorielle Kombination von Nährstoffzugabe und Beleuchtung des Unterwuchses (beides entweder angewendet oder nicht) über zwei Jahre um zu zeigen, dass die Beleuchtung des Grasunterwuchses die Artenvielfalt erhält, trotz des Zusatzes an Nährstoffen. Ausserdem zeige ich, dass die Konkurrenz um Ressourcen im Boden keine messbare Rolle in Bezug auf den Artenverlust spielt. Meine Resultate erklären die besondere Gefahr des übermässigen Nährstoffeintrags für die Pflanzenvielfalt, indem sie die erhöhte asymmetrische Konkurrenz um Licht als den Hauptmechansimus aufzeigen. Die

Ergebnisse betonen ausserdem, dass Düngung kontrolliert werden muss, wenn Pflanzenvielfalt erhalten bleiben soll (Hautier *et al.*, 2009).

In **Kapitel 3**, zeige und teste ich ein einfaches Modell über die Wirt-Parasit Interaktion bei welchem die Wachstumsrate des Parasiten eine Funktion der Wachstumsrate des Wirtes ist. Das Modell sagt voraus, dass das kombinierte Gewicht (die Menge) des Wirt-Parasit Systems immer kleiner ist als das Gewicht des Wirtes allein. Ausserdem sagt das Modell voraus, dass der Parasit eine mittelschwere Virulenz annehmen sollte um seine Leistung zu maximieren, aber dass die optimale Virulenz von den Wachstumseigenschaften des Wirtes abhängt. Um die Hauptannahme des Modells zu testen bestimme ich den Einfluss von *Rhinanthus alectorolophus,* eine weitverbreitete halbparasitische jährliche Pflanze, auf neun häufige europäische Grasarten. Ich zeige, dass die Leistung von *Rhinanthus* stark korreliert ist mit der Wachstumsrate der Wirtspflanzenart, und somit kann ich die Hauptannahme des Modells bestätigen. Aber Gräser unterscheiden sich in Bezug auf ihre Fähigkeit sich gegen Parasitismus zu wehren. Die Parametisierung eines solchen Modells der Wirt-Parasit Beziehung könnte helfen die unterschiedlichen Leistungen von *Rhinanthus* bei verschiedenen Wirten, die unterschiedlichen Einflüsse des Halbparasiten auf Weideland mit unterschiedlicher Produktivität und Unterschiede in der Virulenz zwischen Parasitenpopulationen zu erklären.

In **Kapitel 4**, berichte ich von einem kurzzeitigen Experiment, welches den Einfluss der Körpergrösse von Samenfressern auf das Überleben von tropischen Baumkeimlingen in einem gerodeten Wald in Borneo bestimmt. I manipuliere die Samendichte in Flächen nahe von con- oder heterospezifischen Bäumen und schliesse Samenräuber verschiedener Körpergrösse mit Käfigen von diesen Flächen aus. Ich zeige, dass wenn grössere Samenfresser keinen Zugang zu den Flächen haben, kleinere Räuber das Überleben der Samen von Mutterbäumen reduzieren, was einen Vorteil für die Samen anderer Arten darstellt (das ist der kritische Prozess der Janzen-Connell Hypothese). Dieser Effekt entsteht nicht, wenn grössere Räuber Zugang zu den Samen haben. Meine Ergebnisse zeigen, dass die spezifische Samenwahl der kleinen Predatoren entscheidenden sein kann in Urwäldern, gerodeten Wäldern oder Waldfragmenten wenn grössere Predatoren fehlen.

General introduction

Between 1980 and 2000, Earth experienced dramatic environmental changes (Houghton *et al.*, 2001). It had two of the warmest decades ever recorded (1980s and 1990s) and three intense and persistent El Niño events (1982 to 1983, 1987 to 1988, and 1997 to 1998). Human-induced ecological disruption through climate and other global change, is accelerating species extinction rates, which are 100 to 1000 times greater than pre-human levels (Chapin *et al.*, 1997; Lawton and May, 1995; Pimm *et al.*, 1995), and have the potential to affect ecosystem processes. Vitousek *et al.* (1997) report that human-induced changes in biodiversity and alterations to the structure and functioning of ecosystems are the two most dramatic ecological trends of the past century.

Importance of grasslands

Grasslands cover more than 40 percent of Earth's land area (White and Vanasselt, 2001). They represent the major biome on earth and one of the most productive and diverse of all terrestrial ecosystems (IUCN 1999). Grasslands are found on every continent except Antarctica and are given local names: the prairies in central and North America, the Pampas in South America, the Savannas (or Veldts) in Africa, the Steppes in Eurasia and the Rangelands in Australia (Krishnamurthy, 2003). They represent more than one third of the agricultural land use in Europe and more than two thirds in Switzerland (Smit *et al.*, 2008). Grasslands are of global importance due to ecosystem services humans receive from them including their ability to supply food for domestic livestock, maintain nutrient cycling, store carbon and water, purify soil and water, regulate the weather and climate, protect against disaster (landslide, erosion), provide pollination and scenic beauty (Daily *et al.*, 2000; White and Vanasselt, 2001).

Does biodiversity matter for the functioning of grassland ecosystems?

Or, from a human point of view "Does biodiversity matter for the provision of ecosystem services that humans obtain from grassland ecosystems?" In *The Origin of Species* (Darwin, 1985; see also Hector and Hooper, 2002) Darwin clearly identified that more diverse communities are more productive. He said, "It has been experimentally proved that if a plot of ground be sown with one species of grass, and a similar plot be sown with several distinct genera of grasses, a greater number of plants and a greater weight of dry herbage can thus be raised." Since Darwin, many experiments have been set up to study the effects of terrestrial plant diversity and ecosystems functions (Hector *et al.*, 1999; Naeem *et al.*, 1994; Roscher *et al.*, 2005; Silvertown *et al.*, 2006; Tilman *et al.*, 1997; Tilman *et al.*, 2001; Tilman *et al.*, 1996; van Ruijven and Berendse, 2003). A variety of ecosystem processes were monitored with a focus on biomass production (productivity). All of these experiments have widely proved that biodiversity positively affects the functioning of ecosystems by en-

hancing individual functions such as productivity, nutrient uptake, stability, resilience and invasion resistance (Duffy, 2009; Hooper *et al.*, 2005) and restoration (Callaway *et al.*, 2003; Pimm *et al.*, 1995) as well as multiple functions simultaneously (Duffy *et al.*, 2003; Hector and Bagchi, 2007; Reich *et al.*, 2001; Weigelt *et al.*, 2009). Although these studies have established the importance of biodiversity for the maintenance of ecosystem functions and their capacity to provide goods and services, understanding the causal mechanisms responsible for the maintenance and restoration of plant diversity will be important for the development of effective conservation policies.

Impact of eutrophication and plant diversity loss on grassland functions

The productivity of most grassland is limited by the supply of biologically available nitrogen and phosphorous (Vitousek and Howarth, 1991). Humans are however altering the global cycle of nitrogen and phosphorous via combustion of fossil fuels, utilization of fertilizers, atmospheric deposition and cultivation of nitrogen-fixing legumes (Galloway *et al.*, 1995). In fact, we have more than doubled the pre-industrial rate of supply of nitrogen to terrestrial ecosystems and global phosphorus liberation (Carpenter *et al.*, 1998; Galloway *et al.*, 2004; Tilman *et al.*, 2001; Vitousek *et al.*, 2002) and anthropogenic terrestrial nitrogen inputs are predicted to be about three to four times the pre-industrial rate in 2050 (Tilman, 1999; Tilman *et al.*, 2001). Much of this nitrogen will enter rivers and be carried to near-shore marine ecosystems, but it will also be deposited atmospherically on non-agricultural terrestrial ecosystems.

In grasslands, studies of nutrient deposition (Stevens *et al.*, 2004) and experimental evidence (Crawley *et al.*, 2005; Harpole and Tilman, 2007; Silvertown *et al.*, 2006) show that nutrient enrichment – or eutrophication – increases productivity and biomass accumulation, at least in the short-term (Vitousek and Howarth, 1991) and also generally reduces the biological diversity of affected ecosystems (Gough *et al.*, 2000; Suding *et al.*, 2005). Consequently, nutrient enrichment is recognized as one of the major causes of biodiversity decline in terrestrial ecosystems (Phoenix *et al.*, 2006; Sala *et al.*, 2000; Stevens *et al.*, 2004) and is projected to be one of the three major drivers of biodiversity loss this century (Sala *et al.*, 2000). These changes will have dramatic impacts on the diversity, composition, and functioning of natural ecosystems of the world, and on their ability to provide society with a variety of essential ecosystem services. A mechanistic understanding of the reduction of diversity due to nutrient enhancement is therefore urgently needed to develop strategies that minimize the potential loss of biodiversity.

Mechanisms of plant species coexistence

Niche complementarity and plant species coexistence

To understand the mechanisms responsible of this decline in diversity we have to understand the mechanisms of species coexistence and competitive exclusion. It seems intuitive that every species should have its niche: under a canopy of tall, sun-seeking trees live shade-tolerant plants. According to the *principle of competitive exclusion* (Gause, 1934), based on the Lotka-Volterra competition model (Lotka, 1927; Volterra, 1926), the traditional niche theory asserts that species can co-exist in a community only when they differ from one another in resource uses. These ‘niche complementarity’ models state that differences among species in their requirements for different resources will cause complementary interactions so that a combination of species could obtain more resources than could any species living by itself resulting in higher biomass production.

The best developed mechanistic model of resource-competition is the resource-ratio theory of Tilman, known as Tilman's R* (Tilman, 1982; Tilman, 1988). This model predicts that when two species are limited by a single resource, the best competitor will be the species that can reduce this resource to the lowest level (R*) and should displace the other species. This alone does not allow coexistence but rather competitive exclusion. However, if two species are limited by different resources (or use different niches) then coexistence is possible if each species consumes most of the resource that most strongly limits its growth. The predictions of this theory have been demonstrated in terrestrial ecosystem for soil nitrogen (Harpole and Tilman, 2006; Tilman, 1991; Tilman and Wedin, 1991) and recently for light (Dybzinski and Tilman, 2007; Vojtech *et al.*, 2008; Vojtech *et al.*, 2007), where the species that reduced the limiting resource to the lowest level in monoculture was the best competitor in pairwise mixture. In eutrophied terrestrial plant communities, an increase in the strength of competition for light due to deeper shading following the increase in aboveground productivity caused by eutrophication is thought to be a mechanism of diversity loss following eutrophication (Newman, 1973; Stevens and Carson, 2002; Tilman, 1982).

Unified Neutral Theory

However, the niche differentiation theory has some difficulties in explaining the diversity often observed in specie-rich communities such as tropical forests. There are so many species in such a community (over a thousand tree species in a 52-hectare plot in Borneo, Lambir, Sarawak (Volkov *et al.*, 2005)), can there be so many resource niches for these species? As an alternative to the niche theory, Stephen Hubbell and others put forward an explanation analogous to the neutral theory in population genetics (Hubbell, 1979, 2001, 2005, 2006; Hubbell and Foster, 1983, 1986) where ecological communities are regulated by neutral processes. The *Unified Neutral Theory of Biodiversity and Biogeography* of Hubbell has had surprising success in reproducing realistic community properties - surprising in that key assumptions of the neutral theory (equal probabilities of birth, death, immigration and speciation for different species) seems to contradict the apparently obvious differences between species. Hubbell's theory assumes no difference in rates of birth, death, immigration and speciation among species, and stabilizing factors such as density-dependence are also non-existent in such a community. For example, density-dependence implies that the death rate will increase with the community size, which gives rare species advantages (Armstrong, 1989; Chave and Leigh, 2002). Hubbell claimed that ecological equivalence stands as long as the intensity of density-dependence is equal among species (Hubbell, 2003). But the birth rate of an individual of rare species should be higher than that of a common species if density-dependence works. Hence, density-dependence and ecological equivalence are contradictory.

Negative feedbacks

A classical example of density-dependence is the Janzen-Connell effect (Connell, 1971; Janzen, 1970). This theory proposes that species-specific predators or herbivores concentrate their activities near the parent organism where initial seed and seedling density is high. Reduced survival of conspecific offspring caused by these negative feedbacks increases the probability of establishment of other species ultimately promoting the coexistence of many species. Although Janzen-Connell effects have been widely studied in tropical forests, its importance as a mechanism of coexistence has not been clearly demonstrated (Gilbert, 2005; Hyatt *et al.*, 2003).

Another example of stabilization of fitness differences via negative feedbacks is the differences in host resistance to parasitism. Resistance might induce a growth cost, leading to a trade-off between growth rate and parasite resistance that might promote coexistence. For example, hemiparasites have been shown to reduce grass and legume productivity relative to forbs that promotes coexistence of grassland communities (Bullock and Pywell, 2005; Pywell *et al.*, 2004).

Is there a future for grasslands? Implications for management and conservation

Non-manipulative strategies

During the last 20 years, management procedures have been developed and applied to counteract the negative impact of agriculture on the environment. For example, many European countries, (including Switzerland (Dietschi *et al.*, 2007)) and the United States (Bohlen *et al.*, 2009) apply agri-environment schemes where farmers are paid to adapt the management on parts or all of their farms to the benefit of biodiversity, environment or landscape. Agri-environment schemes play an increasingly important role in The European Union's Common Agricultural Policy (CAP) to support biodiversity and environment in agricultural landscapes. The effectiveness of political incentive payments is controversial in Europe (Hole *et al.*, 2005; Kleijn *et al.*, 2001; Kleijn *et al.*, 2004) but promising in Switzerland (Dietschi *et al.*, 2007; Knop *et al.*, 2006).

Manipulative strategies

Although observational studies find that plant tissue nutrient content, soil pH, and soil extractable inorganic N pools may recover after fertilization stops (Boxman *et al.*, 1995; Power *et al.*, 2006) plant composition and phenology, as well as microbial populations and activity may not (Clark and Tilman, 2008; Nordin *et al.*, 2005; Power *et al.*, 2006; Strengbom *et al.*, 2001). Indeed, litter thickness, litter N content and soil N mineralization rates often remain elevated long after inputs cease, limiting the potential for plant community diversity and composition to recover (Vinton and Burke, 1995). This lack of process recovery likely maintained the persistence of the new community. Thus, manipulative strategies may be required to restore biotic and abiotic conditions favourable to the original community (Bakker and Berendse, 1999; Suding *et al.*, 2004). Moreover, several studies have shown that several interacting factors must be in place for the re-establishment of a plant community following reduction of N inputs (Bullock and Pywell, 2005).

Reducing N availability

Several studies have found evidence of elevated N cycling long after inputs cease (Power *et al.*, 2006; Vinton and Goergen, 2006). Methods have been developed to reduce N availability including sod removal (Berendse *et al.*, 1992), increased frequency of fire (Howe, 1994), or addition of organic carbon (Morgan, 1994). Of these, carbon addition shows the greatest promise for increasing target native species relative to non-target invasive species (Blumenthal, 2003; Eschen *et al.*, 2006). Carbon addition induces microbial immobilization of soil N, reduces availability of soil N, and reduces plant biomass (Blumenthal, 2003; Eschen *et al.*, 2007; Paschke *et al.*, 2000; Torok *et al.*, 2000), often favouring natives over invasives (Blumenthal, 2003; Paschke *et al.*, 2000; Zink and Allen, 1998).

Removal of litter layer

Litter plays a key role in structuring plant communities, through its physical inhibition

of growth, reduction in light levels to the soil, storage of nutrients, and facilitation of elevated pathogen levels (Facelli and Pickett, 1991; Foster and Gross, 1998; Tilman, 1993). Removal of the litter layer, a process normally carried out by fires in herbaceous systems, may be required to open up germination sites, increase light levels at the soil surface, and reduce ecosystem N stores to create favourable competitive conditions (Foster and Gross, 1998; Tilman, 1993).

Increased clipping regime

Chronic disturbance can alter availability and/or spatial distribution of resources (Robertson *et al.*, 1993; Robertson *et al.*, 1988; Rover and Kaiser, 1999), which can in turn strongly influence vegetation pattern, community structure, and diversity in terrestrial ecosystems (Grime, 1979; Huston, 1979; Stevens and Carson, 2002; Tilman, 1984). Clipping regime have been used as chronic disturbance to lower soil nitrogen levels, increase light availability and increase short term species diversity (Fynn *et al.*, 2004; Han *et al.*, 2007).

Reduced seed recruitment limitation

With or without favourable resource and physical conditions in place, a lack of viable propagules either in the seed bank, the existing community, or in the regional species pool could limit the re-establishment of the original community (Willems, 2001; Zobel, 1997). Many herbaceous communities are recruitment limited (Grubb 1977), suggesting that recruitment limitation may be especially severe in species poor communities that result following N enrichment.

Use of hemiparasitic plants

Another restoration technique is the use of naturally occurring hemiparasitic plants of the genus *Rhinanthus*. In particular, *R. alectorolophus* and *R. minor* have shown to be promising tools in restoring grassland diversity (Bullock and Pywell, 2005; Davies *et al.*, 1997; Pywell *et al.*, 2004; Westbury *et al.*, 2006). These species feed off the neighbouring grasses and legumes by tapping into their root network, reducing their competitive vigour and benefiting wildflower species.

Thesis outline

In this thesis, I experimentally manipulate ecosystem properties to test fundamental hypotheses of species coexistence in terrestrial plant communities. Focusing on light availability I first investigate the importance of competition for light in grasslands under conditions of varying productivity and disturbance (Chapter 1) and its consequence for species coexistence and restoration (Chapter 1 and 2). I then focus on negative feedbacks by natural enemies to explore the mechanisms of maintenance of diversity in temperate grasslands (Chapter 3) and tropical forests (Chapter 4).

In **Chapter 1**, I measure light availability in grass monocultures to test how well they predict competitive outcomes in pairwise mixtures under productive, unproductive and disturbed conditions. The results confirm the importance of asymmetric competition for light under productive conditions but not under unproductive or disturbed conditions. My findings support the idea that when ecosystems receive sufficient light plants compete primarily for limiting nutrients – a size-symmetric process – while with ample nutrients plants compete primarily for limiting light (a size-asymmetric process). My data suggest that a possible mechanism by which two restoration tools frequently used in grasslands (frequent cutting and carbon addition to soil) might restore plant diversity is by directly or indirectly increasing light availability to subordinate plants preventing asymmetric competition for light dictating the outcome of competition over the growing season.

In **Chapter 2**, I use a novel manipulation of experimental addition of light to the understory of fertilized grassland communities to test whether fertilization reduces diversity through increased asymmetry in competition for light. Moreover, I measure the strength of belowground competition by comparing transplanted seedlings planted in plastic tubes to reduce belowground competition with transplanted seedlings exposed to full root competition. I provide the first direct experimental demonstration that asymmetric competition for light is a mechanism of plant diversity loss following eutrophication and show that competition for nutrients does not play a role in the loss of diversity.

In **Chapter 3**, I present and test a simple model of the host-parasite interaction in which parasite growth rate is a function of host growth rate. I use size corrected growth rate of nine grass species to test whether they are related to the performance of the hemiparasitic plant *Rhinanthus alectorolophus*. I demonstrate that *Rhinanthus* performance is a simple function of the growth rate of the host grass species. However, *Rhinanthus* biomass on three of the nine grass species differed significantly from the predicted value suggesting that grasses may differ in their resistance to parasitism. Parameterising such models of the host-parasite relationship could help to explain variation in *Rhinanthus* performance on different hosts; variation in the effects of hemiparasites in grasslands of different productivity; and differences in virulence among parasite populations.

In **Chapter 4**, I investigate the separate contribution of small and large seed-predators on tropical tree seed mortality in

logged forest by manipulating seed density and distance to mother tree and comparing seed survival from exclosure cages that excluded seed-predators of different body sizes. I demonstrate that small predators increased seed recruitment of the distant tree confirming that small predators inflict distance-dependent mortality as predicted by the Janzen-Connell hypothesis. However, this effect was cancelled out when larger predators were present. My results suggest that small predators might help maintaining diversity in primary forest, logged forest or forest fragments that are lacking large predators

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Chapter 1

The importance of competition for light depends on productivity and disturbance

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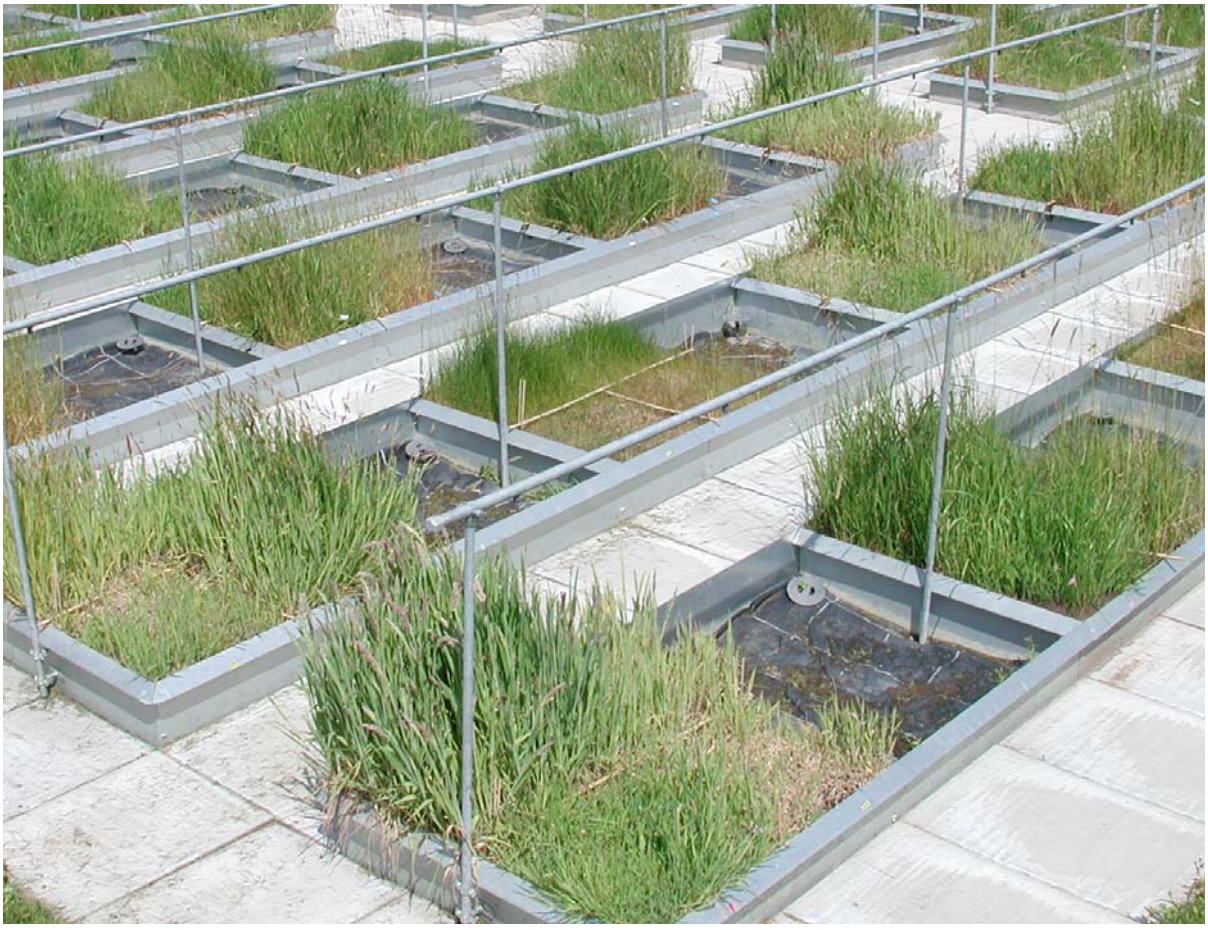

Abstract

Eutrophication is a major cause of biodiversity loss. In grasslands this appears to occur due to asymmetric competition for light following the increases in productivity. Here, we report the results of an experiment with five grass species that tests how well competitive outcomes can be predicted under productive, unproductive and disturbed conditions. Under productive conditions our results confirm earlier success in predicting short-term competitive outcomes based on light interception in monocultures. This effect was maintained but reduced under less productive conditions with competition becoming more symmetric. However, under disturbed conditions, competitive outcomes could not be predicted from differences in light interception in monocultures regardless of productivity. Our results support the idea that competition in grasslands shifts from symmetric to asymmetric as productivity increases but that disturbance destroys this relationship, presumably by reducing competition for light.

Keywords: Disturbance, eutrophication, grassland restoration, sucrose, clipping regime, asymmetric competition, light, nutrients

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Introduction

Humans have greatly enhanced the rate of supply of nutrients worldwide (Galloway *et al.*, 1995; Tilman *et al.*, 2001; Vitousek *et al.*, 1997). In many types of ecosystem this eutrophication causes loss of plant species diversity (Silvertown *et al.*, 2006; Stevens *et al.*, 2004). In grasslands, size asymmetric competition for light has been shown to be a major mechanism of this plant diversity loss (Hautier *et al.*, 2009). Related experiments have shown that under productive conditions the outcome of competition could be predicted from differences in light intercepting ability in monoculture (Vojtech *et al.*, 2008; Vojtech *et al.*, 2007). However, these experiments were limited to productive conditions and were not always able to separate aboveground from belowground competition.

Using a model system of five perennial grass species commonly found in European fertile grasslands, Vojtech *et al.* (2008; 2007) investigated the short-term outcome of competition for light. They performed two experiments under highly fertilized and irrigated conditions where light is assumed to be the limiting resource and competition for light to be important. In one experiment they grew all monocultures, all pairwise mixtures and the full five-species mixtures (see Vojtech *et al.*, 2008). In a companion experiment (Vojtech *et al.*, 2007) they grew one central target plant surrounded by a ring of neighbours of each of the species including itself (i.e. in all possible intraspecific and interspecific pairwise combinations). The level of incident light intercepted in monoculture, a direct measure of resource-reduction ability, was a good predictor of short-term competitive outcomes in pairwise mixtures (Vojtech *et al.*, 2008; Vojtech *et al.*, 2007). In other words, the species that reduced light resource to the lowest level in monoculture was the best competitor in pairwise mixture, consistent with Tilman's resource competition theory (Tilman, 1982; Tilman, 1988). Dybzinsky & Tilman (2007) found similar results over the longer-term: light interception in monocultures of six grass species predicted competitive outcomes in pairwise mixture along a nitrogen gradient. Moreover Vojtech *et al.* (2007) demonstrated that the differences in light intercepting ability

conferred a disproportionate competitive advantage thereby confirming that under productive conditions competition between species for this resource is size asymmetric (Vojtech *et al.*, 2007).

Although their experiments confirmed earlier reports of the importance of competition for light under productive conditions (Nord-Larsen *et al.*, 2006; Schwinning and Weiner, 1998; Weiner, 1990), they did not test for limitation by other potential resources. In particular they did not include measurements of belowground competition. Nevertheless, a related experiment ruled out any detectable role of belowground competition on competitive exclusion under productive conditions (Hautier *et al.*, 2009), suggesting that belowground competition played a limited role in the predictions of competitive outcomes based on light interception abilities. However, neither experiment compares competition under fertilized conditions with that under less productive conditions.

In this paper, we report an extension of the experiment by Vojtech *et al.* (2008) in which we compared the predictions of competitive outcomes under productive, unproductive and disturbed conditions by applying two treatments in a fully-factorial design. Unproductive conditions were obtained by adding a carbon source in the form of sucrose and we increased disturbance of the aboveground canopy by more frequent cutting. Adding a carbon source such as sucrose or sawdust is known to stimulate soil micro-organism immobilisation of nitrogen (Killham, 1994) and competition with plants for nitrate and ammonium (Bardgett *et al.*, 2003; Schmidt *et al.*, 1997). We show that aboveground biomass production was decreased under both unproductive and disturbed conditions thereby increasing the amount of light available in monocultures and reducing the asymmetry of competition for light. However, only when the canopy structure was frequently disturbed could the competitive outcome in pairwise mixture not be predicted from light interception levels in monoculture. Our results demonstrate that size-asymmetric competition for light observed under fertile conditions is reduced at lower productivity and prevented by frequent disturbance of canopy development.

Materials and Methods

Experimental design

The experiment reported here is part of a wider project (Vojtech *et al.*, 2008; Vojtech *et al.*, 2007) about light competition and partitioning in grasslands which uses a model system of five perennial grass species (Poaceae): *Alopecurus pratensis* L., *Anthoxanthum odoratum* L., *Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl, *Festuca rubra* ssp. *commutata* Gaud. (= *Festuca nigrescens* Lam.), *Holcus lanatus* L. (Lauber and Wagner, 2001). The experiment was set up in April 2004 in the experimental garden of the Institute of Environmental Sciences, Zurich (47° 23’ N, 8° 33’ E, and 546 m height a.s.l.) and ran until June 2008. Species were grown in 1 m^2 plots on highly fertile soil (Garden humus, Ricoter, Aarberg, Switzerland) as 5 monocultures, all 10 pairwise mixtures and the single full five-species mixtures in a fully randomized design. Each species combination was replicated five times, yielding a total of 80 plots. Species were sown at a target density of 1000 seeds m^{-2} (corrected based on the results of prior germination trials). Plots were watered daily and weeded on a regular basis. Vojtech *et al*. (2008; 2007) reported the results of the first three years of experiment (2004 to 2006). During 2005 and 2006, plants were continuously fertilized to assure high nutrient amounts with a NPK fertilizer corresponding to 15 g m^{-2} yr^{-1} of nitrogen in five applications during the growth season. Light interception and maximum canopy height were regularly monitored. Aboveground biomass was harvested in

August/September in all three years and in June 2005 and 2006.

In 2007 we divided the plots into four subplots 50 x 50 cm. We applied two treatments in a fully-factorial design to reduce biomass production: addition of sucrose and frequent cutting of the canopy structure. The subplots that did not receive sucrose were continuously fertilized as described above. Plots were watered daily and weeded on a regular basis. The aboveground biomass in the inner 30 x 30 cm of each subplot was harvested to a height of 3 cm in mid June of 2007 and 2008 and late August 2007, sorted to species, dried at 80°C and weighed. The aboveground biomass of the additional cutting treatment in May 2008 was sorted to species, dried at 80°C and weighed. To compare the biomass production of the different treatment at the harvest of June 2008, we combined the measured biomass of the additional cutting (corrected to the inner 30 x 30 cm) to the measured biomass of the harvest of June 2008. Soil cores were collected at the end of the growth season in October 2007 and regularly during the growth season in 2008 and analyzed for nitrate and ammonium concentrations (Labor für Boden- und Umweltanalytik, Thun, Switzerland).

Treatment efficacy

Plots received sucrose in five applications of 500 g m^{-2} $year^{-1}$ during the growth season in 2007 and two applications of 625 g m^{-2} in 2008. The canopy structure was disturbed by increasing the number of harvests from two to four. The two additional harvests were four weeks before the mid- and late-summer cutting typical of European meadows described above.

In monocultures, sucrose addition and frequent cutting significantly decreased biomass production of the harvest of June 2008 ($F_{3,219}$ = 209, P < 0.001; Fig. 1, upper panels) and increased understory light availability measured before harvest (likelihood ratio test: χ^2_3 = 28.7, P < 0.001; Fig. S1), leading to a negative linear relationship between average light availability and biomass production (slope and 95% CI = -0.13 (-0.1 – -0.16); Fig. S2). Averaged over species, plants in the control subplots produced 745 ± 29 g m^{-2} (mean ± s.e.m.) and transmitted 13 ± 6% of the incident light to the understory. Frequent cutting decreased productivity to 427 ± 29 g m^{-2} and increased understory light availability to 58 ± 7%. Sucrose addition decreased productivity to 274 ± 29 g m^{-2} and increased understory light availability to 65 ± 7%. The combination of frequent cutting plus sucrose addition decreased productivity to 207 ± 29 g m^{-2} and increased understory light availability to 81 ± 5%.

Analysis of competition and competitive asymmetry

To investigate the importance of competition for light (cf. Vojtech *et al.* 2007, 2008), we related the biomass ratio of the harvest of June 2008 of each pairwise mixture to the relative difference in light interception of respective species in monoculture at the beginning of the growing season. Both the biomass ratio and the relative difference in light interception were calculated as log-ratios of relative yield or monoculture light interception of the dominant species to the respective value of the subordinate species. We then quantified the relationship between the biomass ratio and relative differences in light interception and tested for symmetry (cf. Vojtech *et al.* 2007, 2008). A slope of 1 reveals symmetric competition and a slope >1 reveals asymmetric competition.

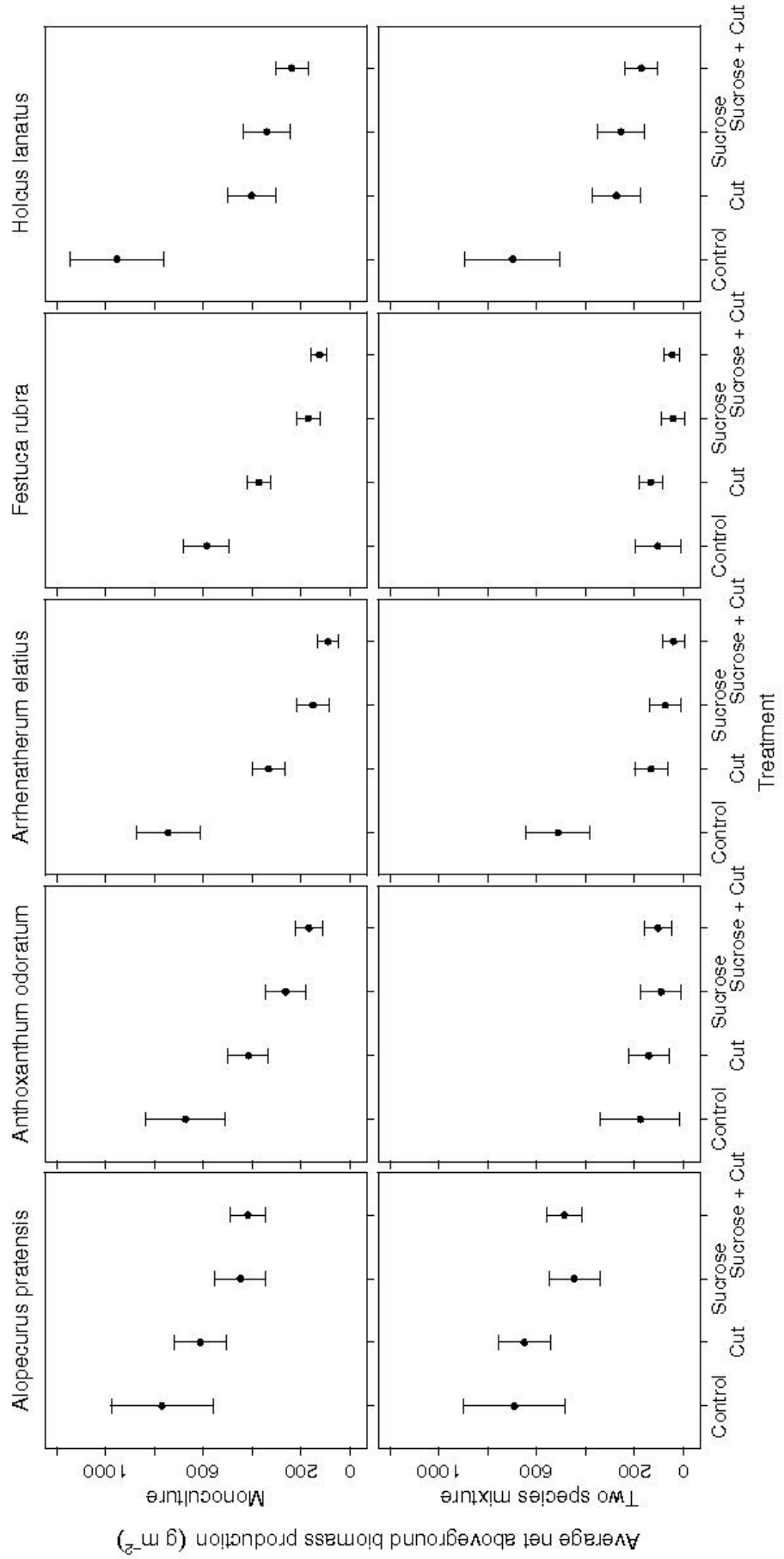

Figure 1. Effects of sucrose addition and frequent cutting on biomass production of five grass species in monoculture and two species mixtures. Biomass was measured at one single harvest in June during the second year of disturbance treatments addition. Points denote treatment means, and the intervals show s.e.m.

Statistical analysis

Biomass production was analysed with mixed-effects models (Pinheiro and Bates, 2000) using the lme function from the nlme library for R 2.9.0 (R Development Core Team, 2009). Treatments, number of species in the mixtures and species identity were treated as fixed effects, and species combinations, plot and subplot were treated as random effects. Since there was heterogeneity in the variance structure between species and treatment we used the varIdent() function to allow each species and each treatment to have a different variance.

Percentage of understory light availability in monocultures measured either at an early stage of vegetation growth or just before the harvest were analysed with generalized mixed-effects models (Gelman and Hill, 2007) using the lmer function from the lme4 library (Bates, 2005) for R 2.9.0. Treatments and species identity were treated as fixed effects, and plot was treated as random effects. In the text and graphs we present estimates of the means from the models with their standard errors (s.e.m.) and linear regression slopes with their 95% confidence intervals (95% CI).

Results

During the second year of our experiment, light intercepting ability in monocultures at the early stage of vegetation growth differed between species (likelihood ratio test: χ^2_4 = 12.9, P = 0.012; Fig. 2) but were not significantly different between treatments (χ^2_3 = 6.4, P = 0.094). Averaged over treatments, *Alopecurus pratensis* intercepted 36% (30 – 42) of the incident light at the beginning of the growing season, *Arrhenatherum elatius* 23% (19 – 28), *Holcus lanatus* 20% (16 – 24), *Anthoxanthum odoratum* 11% (9 – 14) and *Festuca rubra* 9% (7 – 11).

In the productive subplots that were not frequently cut, abundance was strongly related to light intercepting ability at the early stage of vegetation growth (linear regression with 95% confidence intervals in the control subplots, Fig. 3). The slope of the relationship was significantly greater than 1 (slope with 95% CI = 1.61 (1.05 – 2.17)) showing that higher ability to intercept incident light at early stage of vegetation growth conferred a disproportionately large competitive advantage over the growing season. This implies that under the productive conditions of our experiment competition for light was asymmetric, supporting earlier shorter term results from Vojtech *et al.* (2007). After 2 years of sucrose addition, abundance was still related to the ability to intercept incident light despite low productivity (Sucrose, Fig. 3). However the slope of the relationship was not significantly different from 1 (slope with 95% CI = 0.97 (0.53 – 1.42)), showing that when productivity was reduced by immobilisation of nitrogen, competition was symmetric. By contrast, with frequent cutting we found no relationship between the relative competitive effect and differences in light interception regardless of sucrose addition (Cut and Sucrose + Cut, Fig. 3). This result suggests that, regardless of productivity, competitive outcomes were not driven by differences in light interception when the aboveground canopy structure of our experimental communities was disturbed.

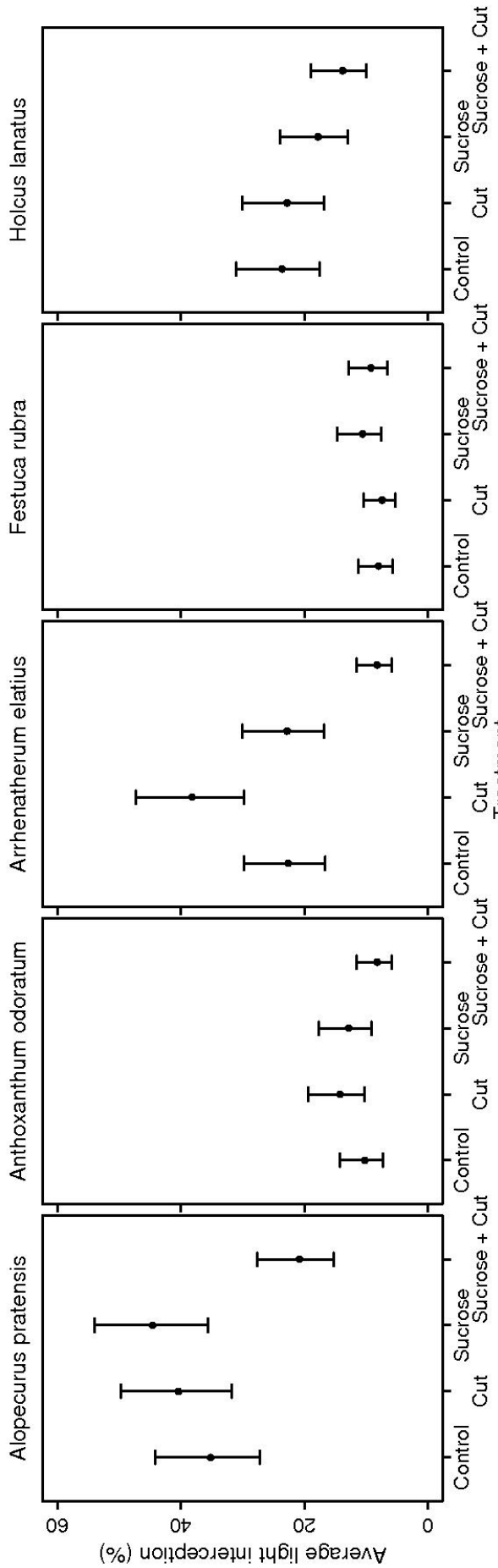

Figure 2. Effects of sucrose addition and frequent cutting on the percentage of incident light intercepted by five grass species in monoculture measured at the early stage of vegetation growth during the second year of treatment addition. Results are shown as in Fig. 1.

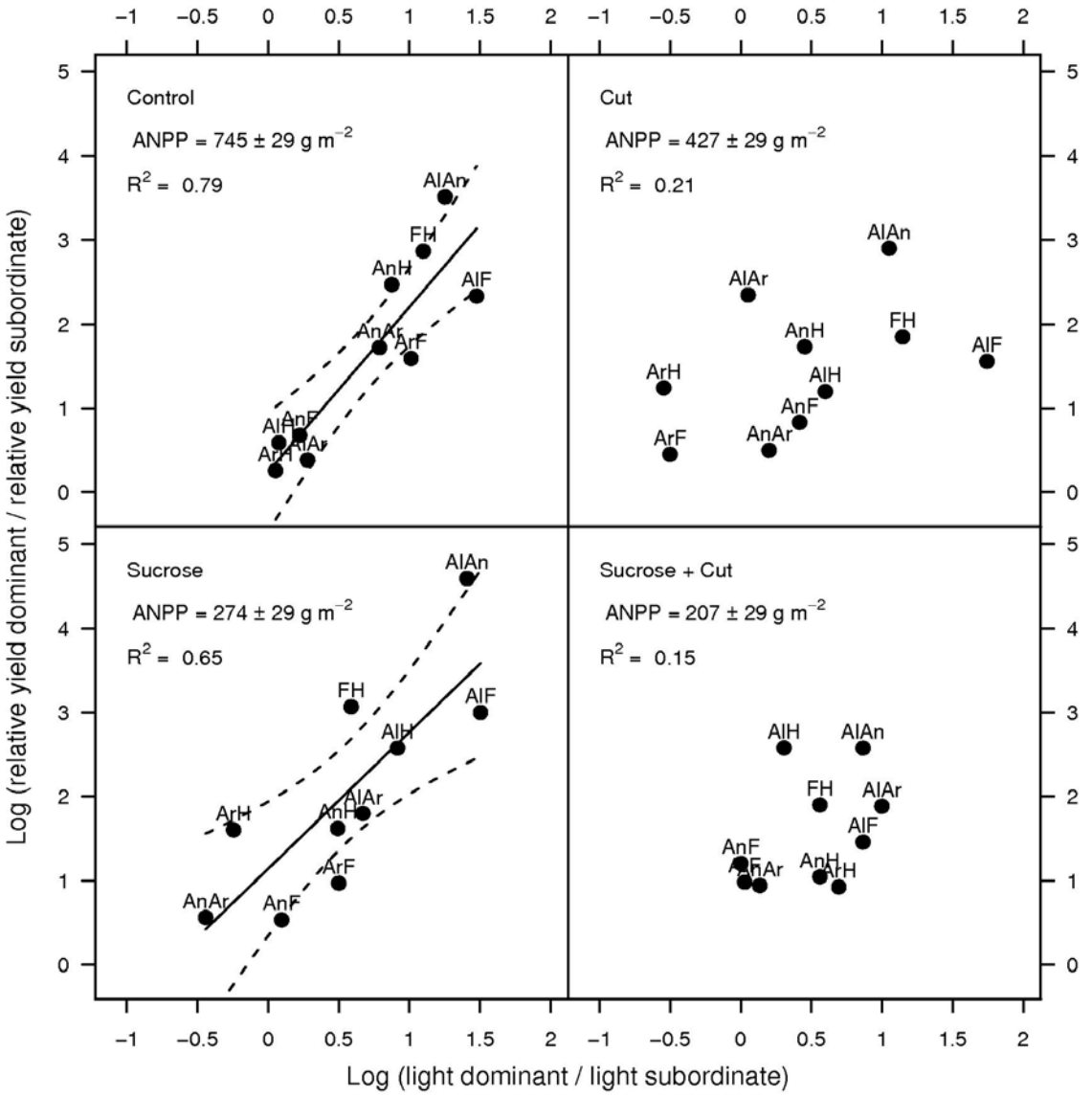

Figure 3. Effects of sucrose addition and frequent cutting on the relationships between the log ratio of relative biomass and the log ratio of relative difference in light interception as measured at the beginning of the growing season. Results are shown as linear regression slopes and 95% CI. Al = *Alopecurus pratensis,* An = *Anthoxanthum odoratum,* Ar = *Arrhenatherum elatius,* F = *Festuca rubra* and H = *Holcus lanatus*. ANPP = average net aboveground biomass production.

Discussion

Vojtech *et al.* (2008; 2007) have shown that under productive conditions, short-term competitive outcomes could be well predicted by differences in the level of incident light intercepted in monoculture. Moreover, Vojtech *et al.* (2007) demonstrated that competition for light is size-asymmetric, confirming earlier studies (Begon, 1984; Nord-Larsen *et al.*, 2006; Schwinning and Weiner, 1998; Weiner, 1986, 1990). In the control subplots of our experiment, after two additional years of fertilization, our study confirm both results: under productive conditions light interception is an excellent predictor of

competitive outcomes and competition for light is asymmetric. For example, species with comparable light intercepting ability at the early stage of vegetation growth– *A. odoratum* versus *F. rubra* or the pairwise combinations of *A. pratensis, A. elatius* and *H. lanatus* (Fig. 2) – produced comparable biomass when grown in pairwise competition (Fig. 3, control). However, when species with low light intercepting abilities – *A. odoratum* and *F. rubra* – were grown with species with higher intercepting abilities – *A. pratensis, A. elatius* and *H. lanatus* – they were disproportionately out-competed in pairwise mixtures (Fig. 3, control). Although we cannot identify light as the only limiting resource, a closely related experiment by Hautier *et al.* (2009) have shown that competition for nutrients had no detectable impact on species exclusion in similar eutrophied conditions. Therefore, our results confirm that under fertilized conditions species with a small initial advantage in light intercepting ability obtain a disproportionate share of this resource and displace poorer light competitors.

Our additional treatments significantly reduced biomass, making it possible to compare the predictions of competitive outcomes under productive condition with unproductive and disturbed conditions. Light intercepting ability measured at the early stage of vegetation growth in monocultures did not differ between treatments (Fig. 2). However, understory light availability measured before harvest was significantly increased by sucrose amendment and frequent cutting (Fig. S1), suggesting that our additional treatments successfully reduced the limitation of light over the growing season. Notably, by contrast with the productive condition, the small initial advantage of dominant species to intercept light at the early stage of vegetation growth did not lead to disproportionate competitive ability under unproductive and disturbed conditions, indicating that our treatments reduced the importance of competition for light (Fig. 3).

Sucrose addition successfully reduced the amount of mineral nitrogen available to plants from 2.3 (± 0.3 g m^{-2}) to 0.9 (± 0.3 g m^{-2}); (Fig. S3). Reduced nutrient availability decreased biomass production and increased light availability over the growing season. Competitive outcomes could still be predicted by differences in light interception at the early stage of vegetation growth despite unproductive condition but competition was more symmetric (Fig. 3, Sucrose). In this case dominant species had a competitive advantage over subordinate species but divided the contested resources in proportion of competitor sizes (Blair, 2001; Casper and Cahill, 1996; Casper and Jackson, 1997; Weiner, 1990). In other words, although the position in the canopy determined dominant and subordinate species within the community, smaller individuals were not at a disadvantage in terms of exploiting resources. There are three possible explanations. First, under less productive conditions competition for light became more symmetric. However, this seems unlikely given that light is a directionally supplied resource. Second, under less productive conditions competition was both above and belowground leading to more symmetric outcome. Third, competition was now primarily for belowground resources but the competitive ability of the five species for nutrients was correlated with their light intercepting ability. It will take further experiments to test these three alternative hypotheses.

Frequent cutting also decreased biomass production and increased light availability over the growing season. Competitive outcomes could not be predicted from light intercepting ability when the canopy structure was disturbed regardless of productivity (Fig. 3, Cut and

Sucrose + Cut). This result shows that the competitive advantage of dominant species over subordinate species disappeared with increased canopy disturbance. This suggests that altering the structure of the canopy layer with frequent cutting prevents the importance of asymmetric competition for light and gives species equal chances to compete for the limiting resources.

Carbon amendments to eutrophied soil (Baer *et al.*, 2004; Blumenthal, 2003; Corbin and D'Antonio, 2004) and clipping regime (Lulow, 2008; Tang *et al.*, 2009) have been used as a restoration tool to lower soil nitrogen levels and increase species diversity. Our results suggest that a possible mechanism is by directly or indirectly increasing light availability to subordinate plants, preventing initial dominance pattern from being maintained and avoiding light intercepting ability dictating the outcome of competition over the growing season.

Our short-term study could not test for limitation by all potential resources and so an additional role of other forms of competition cannot be completely discarded. Nevertheless, altogether our findings support the idea that when ecosystems receive sufficient light plants compete primarily for limiting nutrients, a size-symmetric process that does not lead to disproportionate competitive exclusion, while with ample nutrients plants compete primarily for limiting light, a size-asymmetric process that lead to disproportionate competitive exclusion and plant diversity loss (Hautier *et al.*, 2009). Our results demonstrate that eutrophication exacerbate the importance of asymmetric competition for light relative to initial size differences with dominant plants pre-empting incident light to a threshold under which subordinate species are disproportionately disadvantaged. In this way, initial dominance is maintained during the whole growing season and competitive exclusion can develop very rapidly within a population and have important effects on ecosystem properties.

Acknowledgments

We thank Hossein Moradi, Mélanie Maire and Daniel Trujillo for field assistance. Y.H. is funded by Swiss National Foundation grant 3100A0-107572 to A.H.

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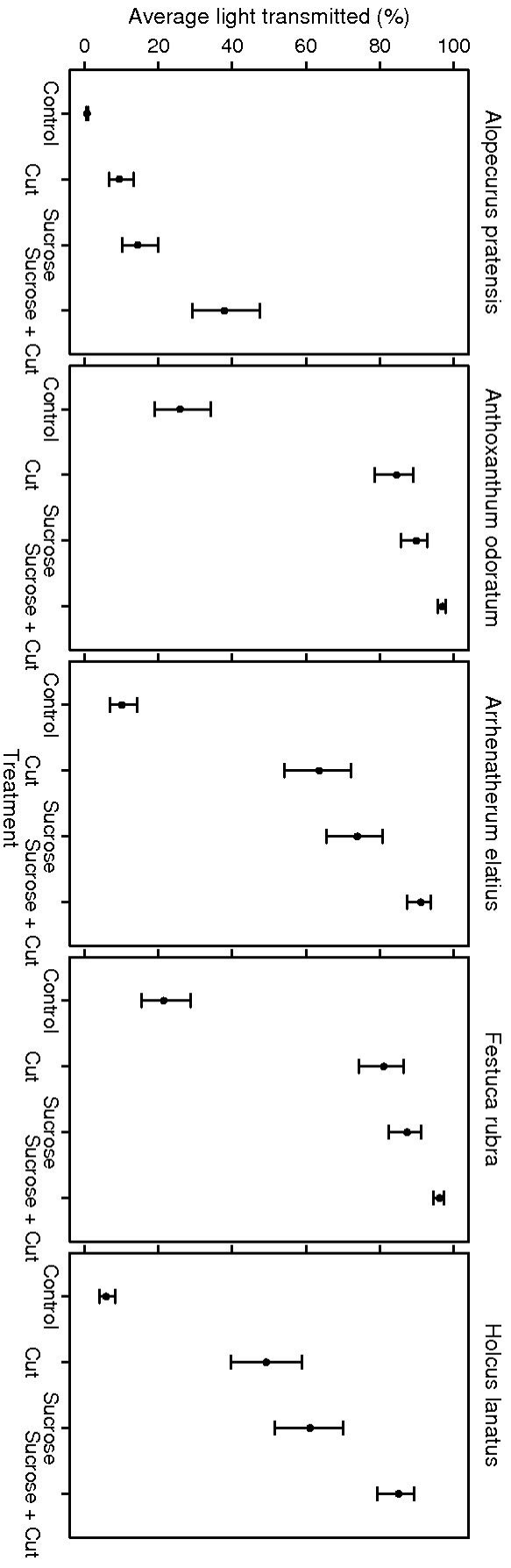


Figure S1. Effects of sucrose addition and frequent cutting on the percentage of incident light transmitted by five grass species in monoculture measured just before the harvest of June during the second year of treatment addition. Results and are shown as in Fig. 1.

Supporting Material

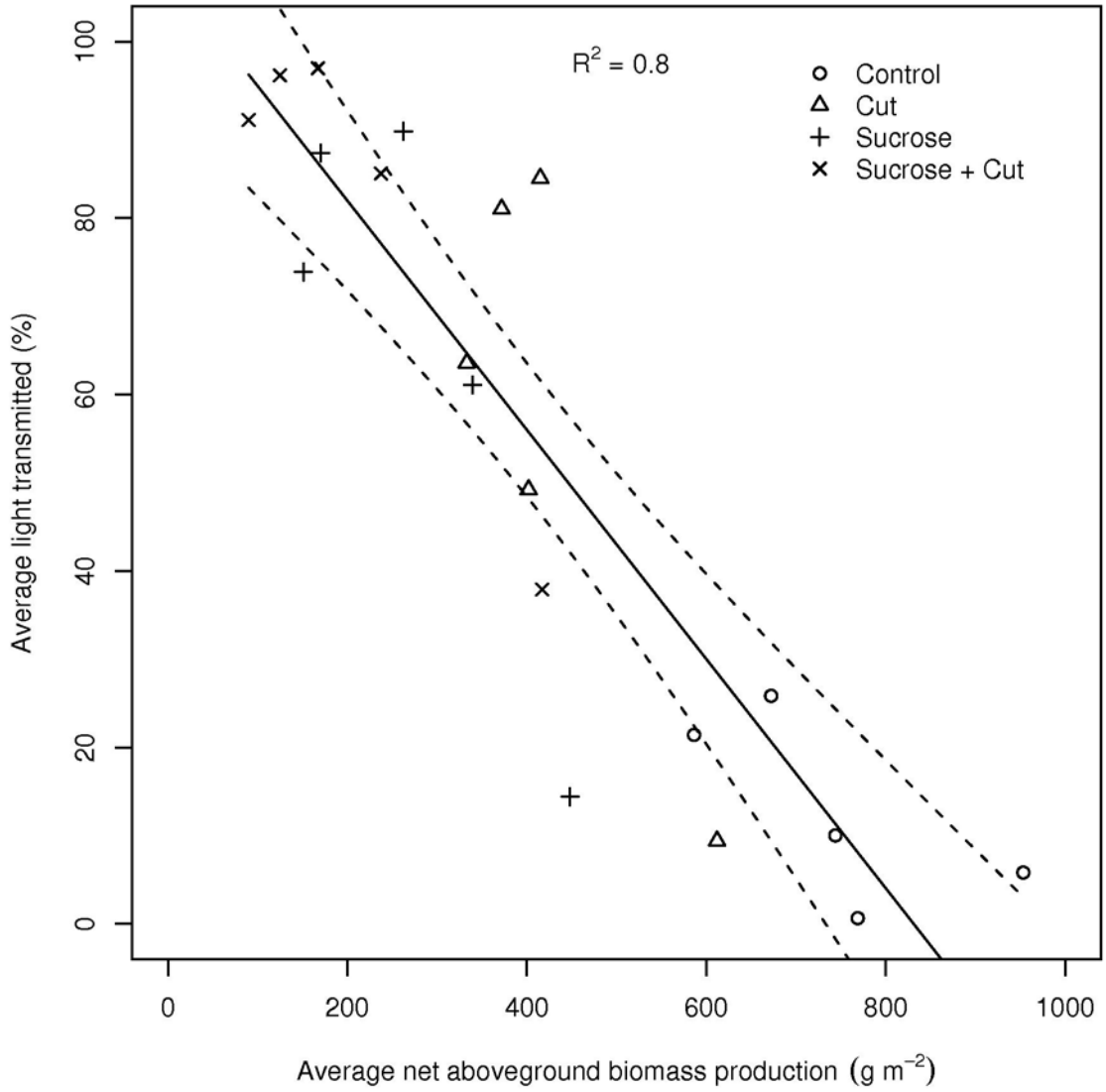


Figure S2. Relationship between the average aboveground biomass production and average light interception in monoculture measured before harvest. Results are shown as in Fig. 3.

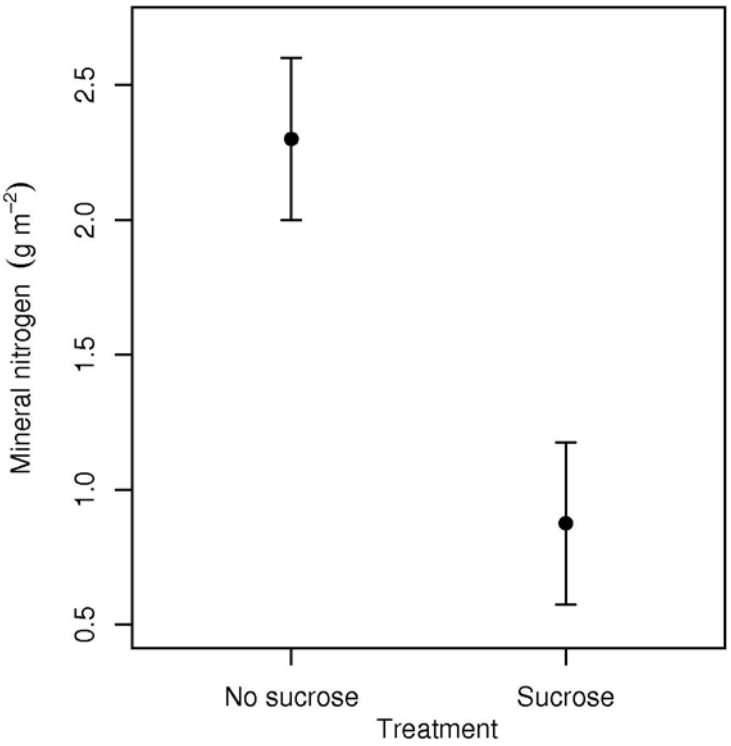


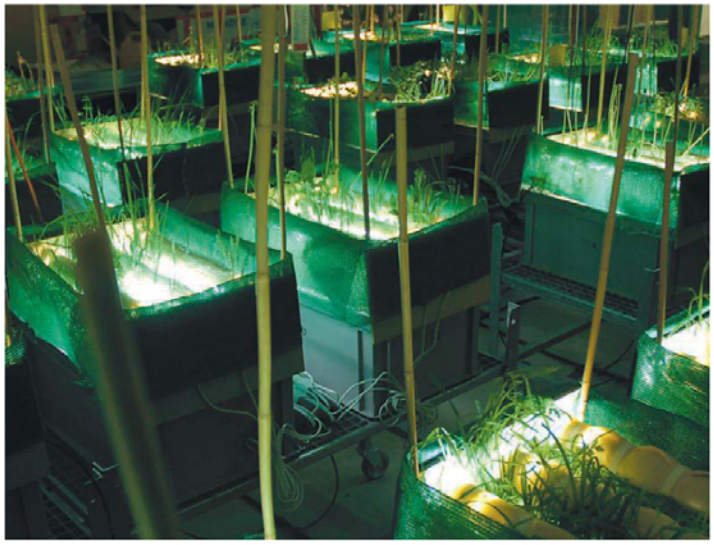
Figure S3. Effect of sucrose addition on the availability of mineral nitrogen. Results and are shown as in Fig. 1.

Chapter 2

Competition for light causes plant biodiversity loss after eutrophication

Yann Hautier, Pascal A. Niklaus & Andy Hector (2009)

***Science*, 324:636-638.**



THIS WEEK IN Science

EDITED BY STELLA HURTLEY

<< Shedding Light on a Problem

Human activities—mainly the application of fertilizers to farmland—have increased the availability of nutrients in terrestrial and aquatic ecosystems. In grasslands, nutrient enrichment reduces plant species diversity, but the mechanisms underlying this loss of biodiversity remain unclear. **Hautier *et al.*** (p. 636) use an experimental manipulation, addition of supplementary light to the grassland understory, which supports the idea that competition for light is the major mechanism of plant diversity loss.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/324/5927/632/DC1
Materials and Methods
SOM Text
Figs. S1 to S6
References

9 December 2008; accepted 18 March 2009
10.1126/science.1169512

Competition for Light Causes Plant Biodiversity Loss After Eutrophication

Yann Hautier,[1]* Pascal A. Niklaus,[1,2] Andy Hector[1]

Human activities have increased the availability of nutrients in terrestrial and aquatic ecosystems. In grasslands, this eutrophication causes loss of plant species diversity, but the mechanism of this loss has been difficult to determine. Using experimental grassland plant communities, we found that addition of light to the grassland understory prevented the loss of biodiversity caused by eutrophication. There was no detectable role for competition for soil resources in diversity loss. Thus, competition for light is a major mechanism of plant diversity loss after eutrophication and explains the particular threat of eutrophication to plant diversity. Our conclusions have implications for grassland management and conservation policy and underscore the need to control nutrient enrichment if plant diversity is to be preserved.

Fertilization experiments (*1*–*4*) and studies of nutrient deposition in terrestrial ecosystems (*5*) show that increases in the availability of nitrogen (*5*, *6*), phosphorus (*7*), and other nutrients—both alone and in combination (*1*, *4*)—usually increase primary productivity and decrease plant diversity. Given that anthropogenic activity has doubled global phosphorus liberation and plant-available nitrogen during the past 50 years (*8*, *9*), and that nutrient inputs are predicted to be one of the three major drivers of biodiversity loss this century (*10*), understanding the mechanisms responsible for diversity loss after eutrophication will be important for the development of effective conservation policies (*11*).

Most of the hypotheses proposed to explain the reduction in plant diversity after eutrophication focus on changes in competition (*12*–*15*). Fertilization may increase the strength of competition generally—that is, both above and below ground (*15*)—or it could increase the strength of aboveground competition for light only: an asymmetric process due to the directional supply of this resource (*13*, *14*). The hypothesis of increased competition for light (*14*) predicts that as productivity increases, availability of light to plants in the understory is reduced, leading to their exclusion by faster-growing or taller species that preempt this directionally supplied resource (*16*, *17*). Surprisingly, 35 years after these alternative hypotheses were suggested, there is no consensus on the role of competition as a mechanism of plant diversity loss after eutrophication (*18*, *19*).

To test whether diversity loss after eutrophication is due to increased competition for light, we added light to the understory of fertilized grassland communities—a manipulation inspired by competition experiments with algae (*20*, *21*). A key advance of our approach relative to earlier work (*22*) is that it restores light to the species in the lower canopy that are thought to decrease in diversity as a result of deeper shading after the increase in aboveground productivity caused by eutrophication. We conducted a glasshouse experiment that combined addition of fertilizer and supplementary light in a fully factorial design. The 32 experimental plant communities were pregrown in the field for 4 years (*23*) before they were extracted with intact soil blocks and moved to the glasshouse. For generality, the communities comprised four different sets of six species (*23*) that had similar levels of diversity and, as we show, responded similarly to the experimental treatments.

Light was added to the understory of each treated community using a system of three fluorescent tubes that were raised as the canopy grew (Fig. 1). Reflectors were placed above the fluorescent tubes to direct light into the understory and to prevent it from shining up onto the underside of the leaves of the taller species. To keep conditions other than light and fertilization as similar as possible, we installed the same system of fluorescent tubes in communities without supplementary light, but in this case reflectors were placed above and below the tubes to form a closed chamber from which the light could not escape. With this system, we were able to experimentally manipulate light in the understory while holding other conditions (such as temperature) constant. Aboveground biomass was harvested twice a year during 2006 and 2007 to coincide with the cutting regimes typical of European meadows, and other key variables (including belowground biomass production, canopy height, availability of light in the understory, soil pH, and plant diversity) were regularly monitored (*24*).

After 2 years of treatment, fertilization had increased net aboveground biomass production and decreased diversity (*24*). During the second year, fertilization significantly increased production from an average of 356 ± 39 g m^{-2} (mean ± SEM) per harvest in the control communities to 450 ± 39 g m^{-2} in the fertilized treatment (Fig. 2A and table S1). The percentage of photosynthetically active radiation in the understory of the fertilized plots (5 ± 4%) was significantly lower than for the controls (13 ± 4%) (Fig. 2B). Notably, when increased production was accompanied by decreased light in the understory, fertilization significantly reduced species richness (Fig. 2C): On average, 2.6 species were lost in the fertilization treatment relative to the control, around one-third of the original species richness. This loss of diversity after eutrophication is consistent with longer-term field studies (*1*, *5*).

When applied together with fertilization, the additional understory light compensated for the increased shading caused by the greater aboveground biomass production and generated levels of understory light (12 ± 4%) that were indistinguishable from those in the control plots (13 ± 4%) (Fig. 2B and table S1). Supplementing understory light in the fertilization treatment to levels similar to the control plots prevented the loss of

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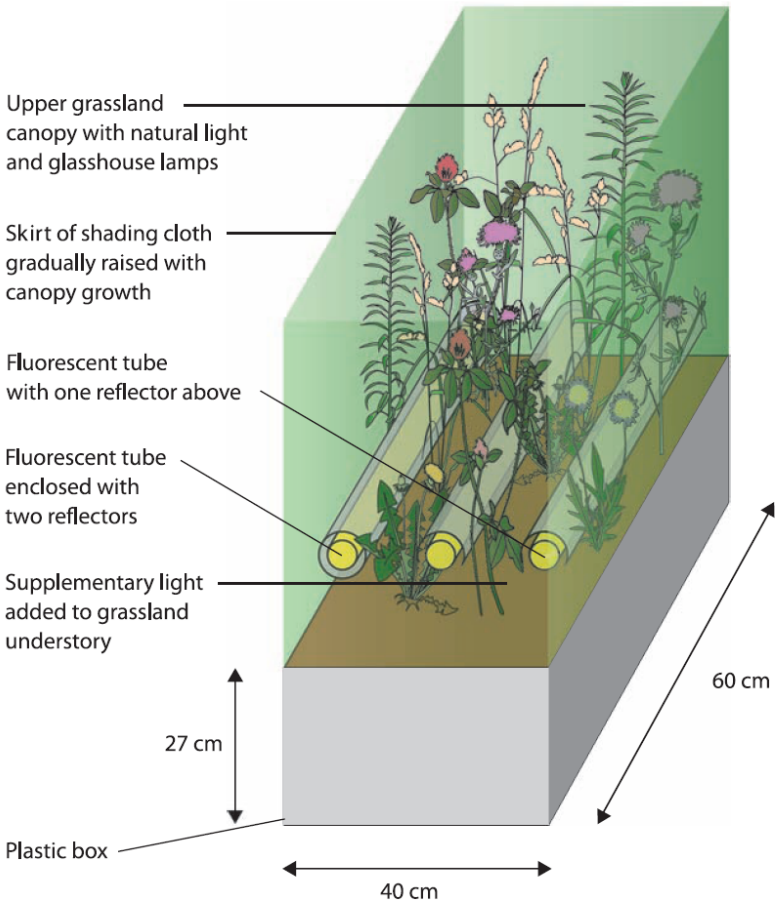

Fig. 1. Schematic representation of the experimental understory light addition. To save space, two open lights and one closed light are shown in the same experimental unit. The four treatment combinations were "control" (unfertilized, closed lights), "fertilization" (fertilized, closed lights), "light" (unfertilized, open lights), and "fertilization + light" (fertilized, open lights). For generality these four treatments were applied to four different plant communities, with each combination replicated twice ($n = 4 \times 4 \times 2 = 32$).

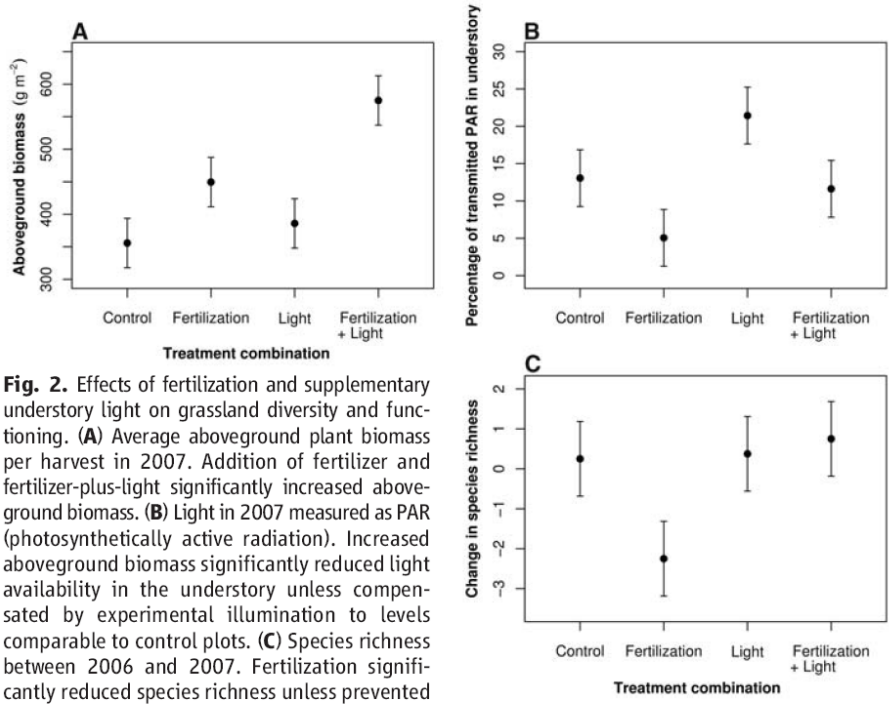

Fig. 2. Effects of fertilization and supplementary understory light on grassland diversity and functioning. (**A**) Average aboveground plant biomass per harvest in 2007. Addition of fertilizer and fertilizer-plus-light significantly increased aboveground biomass. (**B**) Light in 2007 measured as PAR (photosynthetically active radiation). Increased aboveground biomass significantly reduced light availability in the understory unless compensated by experimental illumination to levels comparable to control plots. (**C**) Species richness between 2006 and 2007. Fertilization significantly reduced species richness unless prevented by the addition of supplementary light to the understory. Points denote treatment means, and the intervals show least significant differences (treatments with nonoverlapping intervals are significantly different at $P = 0.05$).

species and maintained comparable levels of diversity (Fig. 2C). This result was general across the four different plant communities used in the experiment; the variance component for the different species mixtures accounted for only 10% of the total of the summed variance components and was nonsignificant (likelihood ratio test: log likelihood = 1.05; $\chi^2 = 2.10$; $P = 0.15$). By mitigating the loss of diversity caused by fertilization, this result supports the hypothesis that increased competition for light was the mechanism responsible for the decline in species richness after eutrophication.

Our communities experienced species turnover that resulted from the loss of resident species and the gain of new species from the seed bank. As in several previous studies (*25*–*27*), the decrease in diversity caused by fertilization was due mainly to a decline in the numbers of species gained (Fig. 3), from 3.2 in the controls to 1.6 in the fertilized plots (table S2). This result was also consistent across the four nonoverlapping communities used in our experiment: The variance component for the different species mixtures only accounted for 2.5% of the total of the summed variance components and was nonsignificant (likelihood ratio test: log likelihood = 0.81; $\chi^2 = 1.61$; $P = 0.20$). There was a marginally significant bias against the establishment of short-statured

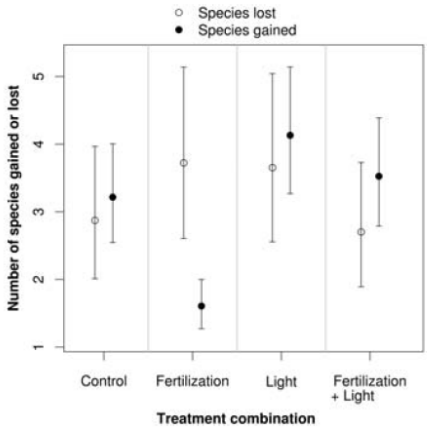

Fig. 3. Species turnover. Decreased diversity in fertilized plots was mainly caused by reduced numbers of species gained. Results are shown as in Fig. 2.

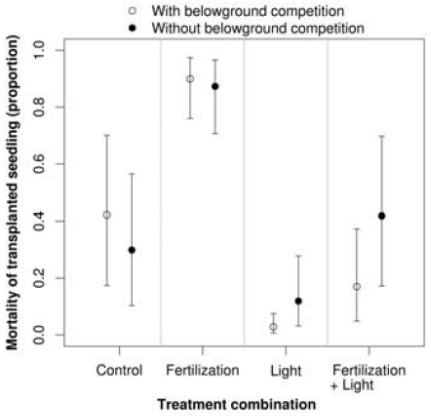

Fig. 4. Seedling mortality. Fertilization significantly increased seedling mortality. Removing belowground competition had little impact on seedling mortality, which suggests that competition for soil resources plays no detectable role in diversity loss. Results are shown as in Fig. 2.

perennial grasses and forbs, but the overall response was not driven by particular species (*24*).

Our understory light addition treatment also had consequences for ecosystem functioning. Net aboveground biomass production in the controls was limited by nutrients (although we cannot exclude light limitation of the taller species as well) because it was increased by fertilization (Fig. 2A and table S1). Without fertilization, the productivity of plants in the understory was not light-limited, because supplementary light had no effect when applied to unfertilized communities (Fig. 2A). However, the productivity of plants in the understory of the fertilization treatment was light-limited, because in fertilized communities the additional light increased average net aboveground production per harvest to 575 ± 39 g m^{-2} (Fig. 2A). These responses suggest colimitation of productivity by light and nutrients, where the taller species are nutrient-limited while understory species in the fertilization treatment are light-limited. More generally, our results suggest that productivity of the upper canopy and understory can be limited by different factors as a result of the directional supply of light.

Species loss could be due to increased competition both above and below ground (*15*). To address this possibility, in the second year of the glasshouse experiment we added seedlings of two species not originally present to the 32 experimental communities to measure the strength of belowground competition. Transplanted seedlings planted in plastic tubes to reduce belowground competition were compared with seedlings exposed to full root competition. The results were consistent with competition for light as the main mechanism of diversity loss: When grown without root exclusion tubes (that is, with belowground competition), seedling mortality (as a proportion) strongly increased with nutrient addition from 0.29 to 0.87, but was comparable to control plots when fertilization occurred together with understory lighting (Fig. 4 and table S3A). The results provided no support for a role of belowground competition in the loss of biodiversity (table S4): Removing belowground competition from fertilized plots had no detectable impact on seedling mortality (table S3B) or seedling biomass (change in biomass = 0.3 g, 95% confidence interval = –1.0 to 1.4).

Although other processes can also contribute to diversity loss, there was no evidence that they were important in our study. Fertilization can reduce grassland diversity through acidification (*2*) or through the accumulation of plant litter (*25*, *26*, *28*, *29*). However, we found no detectable differences in pH after fertilization (fig. S1 and table S5). There was also little buildup of litter during our experiment, which suggests that the negative effects of increased aboveground productivity might have strengthened in the longer term if litter accumulation had occurred.

Together, our results are consistent with increased competition for light as a major mechanism of diversity loss after eutrophication of grassland communities. Fertilization increased productivity and canopy height, and led to reduced light in the understory. In turn, this led to a reduction in diversity, particularly of low-statured perennial grasses and forbs, mainly through reduced recruitment. Other mechanisms also cause loss of plant diversity, but they played no detectable role in our case. Supplementing levels of understory light in fertilized communities reduced competition for light, sustained seedling establishment, and maintained plant diversity despite the additional nutrient inputs.

Some earlier studies (*30*) have demonstrated the importance of competition for light indirectly by tying back the vegetation. Our results advance a long-running debate in community ecology by providing a direct experimental demonstration of the importance of asymmetric competition for light as a mechanism of plant diversity loss. More generally, our work explains and emphasizes the need to develop conservation policies and management procedures that prevent eutrophication if biodiversity is to be conserved.

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Supporting Online Material

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Materials and Methods
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Supporting Online Material for

Competition for Light Causes Plant Biodiversity Loss After Eutrophication

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Supporting Material

Materials and Methods

Experimental design. We used pre-established 4 year old communities from an existing experiment (*S1*). For generality, the communities consisted of four different 6-species mixtures containing grasses, legumes and forbs (Table S6). In September 2005, 32 soil-plant communities, each 60 x 40 x 27 cm were extracted from this field and transferred to an experimental glasshouse. Weeding of the unsown species ensured that the maximum richness at the time of extraction was 6 species and the realized richness of the 4 communities ranged between 3.1 ± 0.83 and 5.9 ± 0.35 species (mean ± s.d.). Plant communities with intact soil blocks were placed in plastic boxes with perforated bottoms. Communities were insulated with expanded polystyrene and installed in the garden of the University of Zurich for the winters between 2005/2006 and between 2006/2007. In April 2006, communities were transferred to a shaded compartment of an experimental glasshouse of the University of Zurich (43°23'N, 8°33'E, altitude 549 m a.s.l.). Communities were watered daily throughout the growing season to keep them moist and were not weeded. After weeding stopped, recruitment of species from the seed bank increased diversity to an average of 7.7 (s.d. = 2.0) species per 0.24 m^2 in 2006 before treatment effects emerged in 2007 and reduced the richness of fertilized communities. Each of the 32 communities was harvested twice a year for two years to coincide with the cutting typical of European meadows. Beginning in April 2006, we applied a fully-factorial combination of nutrient addition and supplementary understory illumination (both either applied or not applied) for two years. The four treatment combinations applied to four different species mixtures, each replicated twice, produced 32 experimental units in total.

Fertilization. In order to reproduce the loss of plant species diversity that usually follows fertilization in the field, we applied a mixture of nutrient commonly used in agriculture in Switzerland (following http://www.landor.ch/fra/3960.aspx?artNr=16612). Fertilizer was supplied in dry form in 4 applications over the growing season at rates (g per m^2 per year) of 15 N, 3.5 P and a cation mix of 6 K, 1.5 Mg, 2.25 Na and 2.25 S (Nitrolplus, Landi, Switzerland) in 2006. Because this only marginally increased biomass, in 2007 fertilization was increased to 20 N, 5 P and a cation mix of 8 K, 2 Mg, 3 Na and 3 S. N was supplied as NH_4 and NO_3 in equal proportions, P as P_2O_5, K as KCl and Mg and S as $MgCO_3$ and $MgSO_4$.

Light addition to the understory. Understory illumination was applied continuously with three parallel fluorescent tubes and reflectors placed in parallel and horizontally above each community at an average height of 15 cm over the soil. The fluorescent tubes (24 W, 6500 K, T5 HO, OSRAM, Winterthur, Switzerland) were 55 cm long and 1.6 cm diameter and produced a light spectrum close to that of the main glasshouse lamps which were specialized for plant growth (400 W, 6500 K, Metal Halide Retrofit from Mercury). We surrounded the supplementary lighting units with a metal grid (1 cm mesh) and a plastic wrap to prevent contact of plants with the fluorescent tubes. The increase in temperature (0.9 °C, s.d. = 0.6) due to the additional light was relatively small and there was no significant difference in the temperature around the fluorescent tubes between treatments with and without understory light (Fig. S2; Table S7, 95% CI = -0.3 – 0.4), that is between the open and closed fluorescent tubes; thus controlling for any potential effects of increased

temperature by equalizing it across treatments. To protect the vegetation, all reflectors were covered with foam (0.5 cm thick). Moreover, eight rotating fans were placed regularly in the glasshouse to disperse the heat throughout the climate-controlled compartment. Both the glasshouse light and the supplementary understory light were on a 14 hour regime. To mimic surrounding vegetation, skirts of 63% shading clothes were placed around all communities and raised to keep pace with the canopy growth (2007 only).

Measurements. We measured aboveground plant biomass production and species composition at peak biomass in early June and September 2006 and 2007 by clipping the entire communities at a height of 2 cm, sorting to species, drying to constant mass in ovens and weighing. The annual aboveground net primary productivity of plant communities within our experimental glasshouse ranged from 305 to 1501 g m-2, which is in the range of those observed for European grasslands (150 to >1500 g m-2) (*S2*). A species was considered lost from a plot if it was present in a harvest in 2006 but absent in the same harvest in 2007 and gained if it was absent in 2006 but present in 2007. Species were classified into 6 groups as being either annual or perennial grasses, legumes or forbs, and into 4 groups according to whether their canopies were basal or leafy and taller or shorter than 30 cm. Species gains and losses were calculated per group by comparing the composition of each plot in 2006 *vs* 2007. The percentage of transmitted photosynthetically active radiation (PAR) reaching the soil surface was measured before cutting using a ceptometer (Sunscan, Delta-T Devices, Cambridge, UK) taking 3 replicate readings across each plot. In 2007, we used root-ingrowth cores (PVC drilled tube of 5 cm diameter and 25 cm deep (*S3*) buried at an angle of 45° in the soil of each community to estimate belowground root productivity. Ingrowth cores were filled with root-free soil sieved (1 cm mesh) during the collection of communities and stored at 4°C. At both peak growth and peak biomass, cores were extracted and roots were separated (sieve, 1 mm mesh), washed to remove soil residue, dried and weighed. In 2007, soil samples were collected at both peak growth and peak biomass, sieved (1 mm mesh) and analyzed for pH (Labor für Boden- und Umweltanalytik, Thun, Switzerland).

Transplanted seedlings. Two-week old seedlings of *Rumex acetosella* and *Plantago media* were transplanted at the beginning of April and middle of June 2007 (following harvest) and grown with or without belowground interspecific competition. Planting holes were filled with root-free soil. Seedling mortality was recorded before communities clipping.

Analysis. We used generalized linear mixed-effects models (*S4*) since our design includes fixed and random effects and our responses include variables with normal and non-normal error distributions. Generalized linear mixed-effects models (GLMMs) (*S5*-*S8*) are generalized linear model that include random effects. The GLMM analyses were implemented, using restricted maximum likelihood, with the lmer function from the lme4 library (*S5*) for R 2.8.0 (*S8*). In the text we present estimates of the means from the GLMMs with their standard errors and in the graphs estimates are given with interval bars to indicate least significant differences (l.s.d.) at P = 0.05 (treatments with non-overlapping intervals are significantly different). Data that were analysed using normal error distribution included productivity, light levels, changes in diversity, canopy height, root-shoot ratios and pH. Data with non-normal error distributions included seedling mortality, which was analysed with a binomial error distribution, and species turnover, which was analysed with a Poisson error distribution. For the analysis of the main fertilization and light addition factorial design, the fertilization and light treatments were treated as fixed effects, and species pools, plots (the 32 individual soil-plant communities) and harvests (two repeated measures per plot within a given year, with years analysed separately) were treated as random effects. In the analysis of the performance of the transplanted seedlings, the fertilization, light addition and root exclusion tube treatments were treated as fixed effects and species pools, plots, harvests and

species identity of the transplanted seedlings were treated as random effects. Random effects for the interaction between species pools and the fertilization and light treatments were very small and non-significant and were excluded during the model building process.

Supporting text

First year result In the first year of our experiment nutrient addition increased above-ground production marginally from 427 ± 162 g m^{-2} per harvest (mean ± s.e.m.) for the control communities to 496 ± 162 g m^{-2} for the fertilized treatment (Table S1). Levels of light in the understory of the fertilized plots (12 ± 8%) were similar to the control plots (13 ± 8%) with no significant difference between the two (Table S1). This was probably due to a lack of surrounding vegetation which allowed the vegetation to spread out and light to penetrate into the experimental communities from the side. Levels of diversity in the fertilized plots (7.6 ± 1.1) were also very similar to those in the control communities (7.1 ± 1.1) with no significant change despite the increased levels of productivity (Table S1), which we hypothesized was probably due to the failure of the fertilization treatment to reduce light availability in the understory during the first year.

Species traits and species loss The decline in species richness in the nutrient addition treatment was mainly due to reduced gains of perennial grass species (95% CI = -1.2 – 0.05) and perennial forbs (95% CI = -2.3 – 0.09) that were both marginally significant. Gains and losses of annual grasses, annual forbs and annual and perennial legumes were independent of productivity. Plants with basal leaves and lower than 30 cm in height also had marginally significantly reduced gains in the fertilized treatment relative to the control (95% CI = -0.1 – 1.2). Hence, most of the changes in species richness with nutrient addition were driven by lower colonization (including from seed bank) of low-growing species of perennial grasses and forbs, while exclusion of established species was not affected.

The role of belowground competition: root-shoot ratios The results provided no support for a role of belowground competition in the loss of biodiversity: removing below-ground competition from fertilized plots had no impact on seedling mortality. Lack of effects of fertilization on community root-shoot ratio, which is presumably related to of the strength of below-ground competition, supports this observation (Fig. S3).

Supporting figures

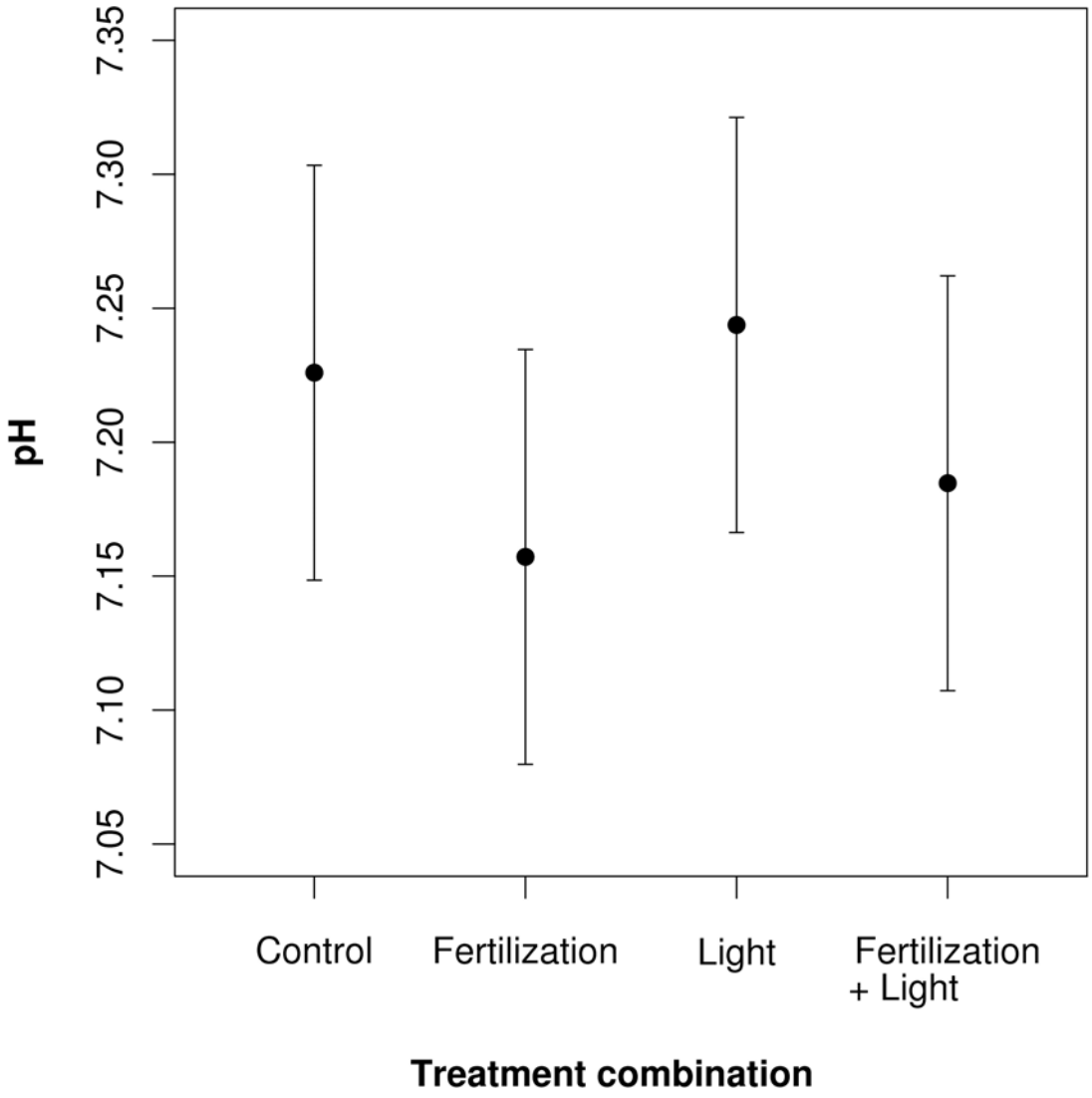

Figure S1. Effects of fertilization and supplementary understory light on pH in 2007. There was little variation in pH and no detectable differences following fertilization. Results are shown as means ± l.s.d.

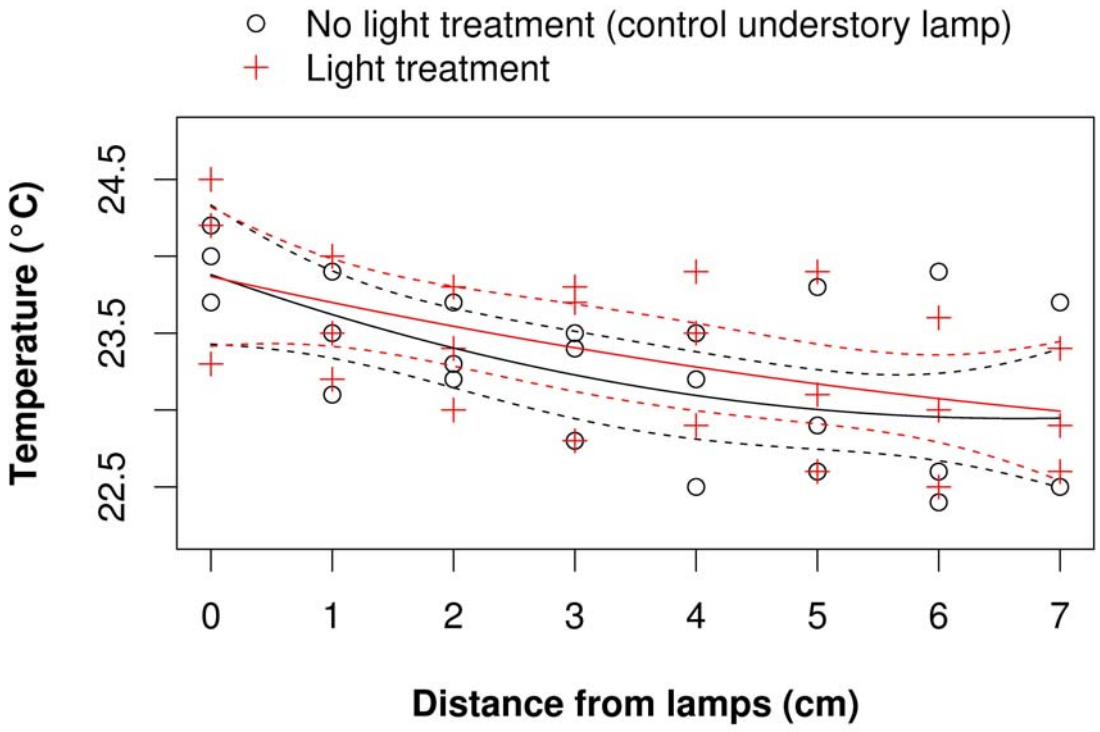

Figure S2. Effects of the supplementary understory light treatment (with/without understory light) on the temperature around the lamps. Solid lines are regression slopes and dotted lines represent the 95% CI for the regression lines.

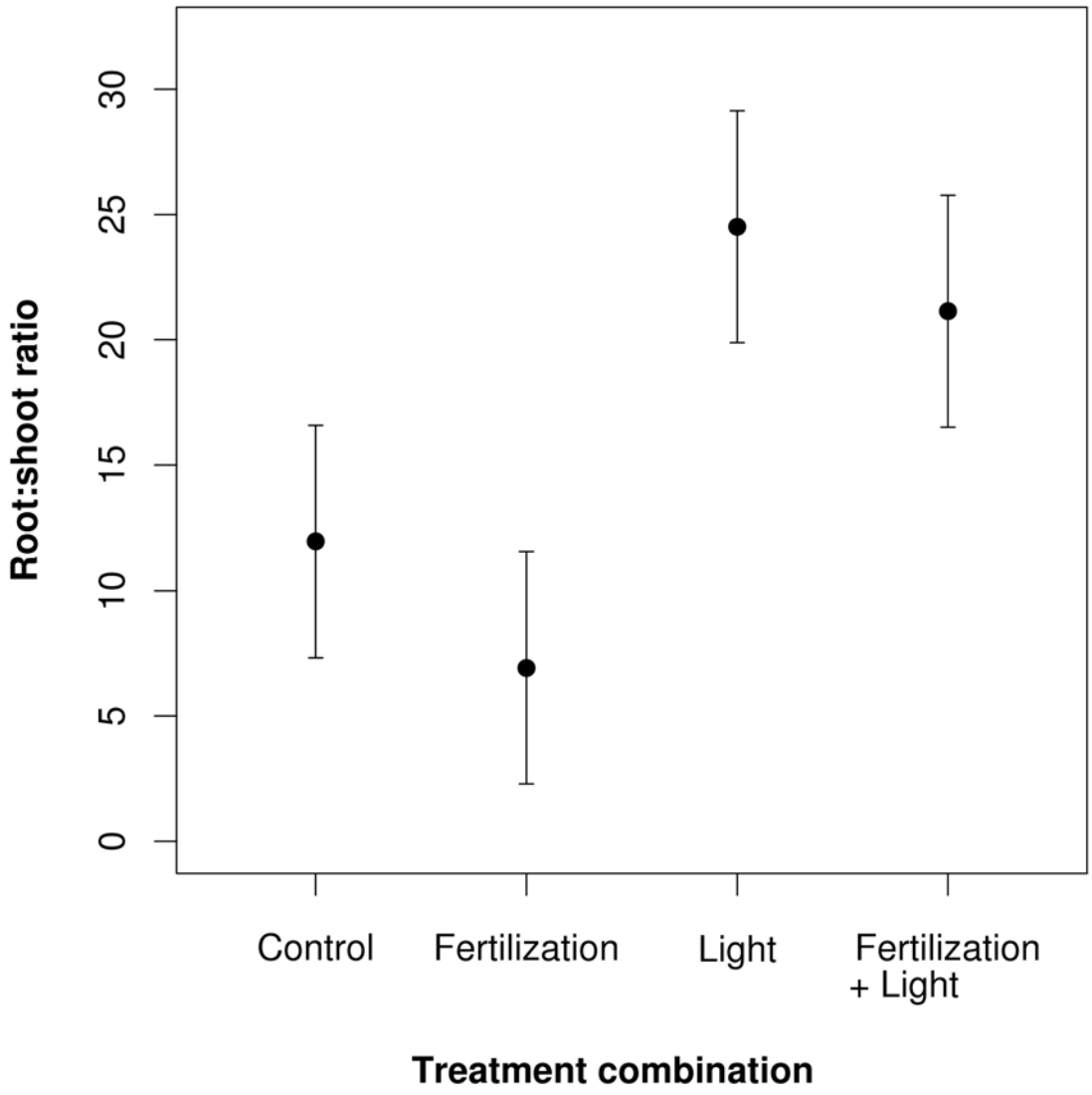

Figure S3. Effects of fertilization and supplementary understory light on biomass allocation in 2007. Additional light increased root allocation. Results are shown as means ± l.s.d.

Supporting tables

Table S1. Effect (productivity, percentage of transmitted light at ground level (PAR) in 2006 and in 2007, species richness in 2006, and changes in species number between 2006 and 2007) of experimental fertilization and supplementary understory light. Results are shown as mean and 95% CI.

2006	Productivity			PAR			Species richness in 2006		
Source	Effect	2.5%	97.5%	Effect	2.5%	97.5%	Effect	2.5%	97.5%
Control	427			13			7.1		
Fertilization	*69*	-10	146	*-1*	-10	7	*0.5*	-0.8	1.6
Light	*-21*	-96	60	*2*	-6	10	*1.1*	-0.1	2.4
Fertilization + Light	*77*	-4	153	*2*	-6	10	*1.0*	-0.2	2.3

2007	Productivity			PAR			Change in species richness between 2006 and 2007		
Source	Effect	2.5%	97.5%	Effect	2.5%	97.5%	Effect	2.5%	97.5%
Control	356			13			0.3		
Fertilization	*94*	22	166	*-8*	-16	-0.5	*-2.6*	-4.4	-0.6
Light	*30*	-43	103	*8*	0.5	16	*0.1*	-1.78	2.0
Fertilization + Light	*219*	147	291	*-1*	-9	6	*0.5*	-1.4	2.4

The effects are reported as the value for the control and the differences (in italics) between control and the other treatments.

Table S2. Effect (species gained and lost between 2006 and 2007) of experimental fertilization and supplementary understory light. Results are shown as mean and 95% CI.

	Gain			Loss		
Source	Effect	2.5%	97.5%	Effect	2.5%	97.5%
Control	1.2			1.1		
Fertilization	*-0.7*	-1.2	-0.2	*0.3*	-0.1	0.6
Light	*0.3*	-0.1	0.6	*0.2*	-0.1	0.6
Fertilization + Light	*0.1*	-0.3	0.5	*-0.1*	-0.5	0.3

The effects are reported as the value for the control and the differences (in italics) between control and the other treatments

Table S3. Effect (mortality of transplanted seedling) A. Of experimental fertilization and supplementary understory light without root exclusion tube in 2007. B. Of experimental fertilization without or without root exclusion tube in 2007. Results are shown as mean and 95% CI.

A.		Seedling mortality		
Source	Root exclusion tube	Effect	2.5%	97.5%
Control	No tube	-0.9		
Fertilization	No tube	*2.8*	1.3	3.5
Light	No tube	*-1.1*	-2.6	0.1
Fertilization*Light	No tube	*0.5*	-0.5	1.3

The effects are reported as the value for the control and the differences (in italics) between control and the other treatments.

B.		Seedling mortality		
Source	Root exclusion tube	Effect	2.5%	97.5%
Fertlization	Tube	2.2		
Fertlization	No tube	*-0.3*	-1.7	1.2

The effects are reported as the mean of seedling mortality with root exclusion tube and the difference (in italic) between the mean of seedling mortality with root exclusion tube and the mean of seedling mortality without root exclusion tube.

Table S4. Transplanted seedling mortality: model comparison for the two alternative hypotheses. Table shows the output from the GLMM statistical analysis giving likelihood ratio tests, information criteria values (AIC), model probabilities (ωi) and evidence ratios (*E*) comparing the models using the AIC (AICc produces qualitatively identical results). There was no main effect of the root exclusion tube (below) or interaction with the fertilization treatment and hence no support for a role of belowground competition in diversity loss following fertilization.

Model	Df	AIC	logLik	Chisq	Chi Df	P	ωi	*E*
Intercept	5	293	-142				0.000	327747.9
Fert	6	286	-137	9.5	1	0.0021	0.000	7903.0
Fert + Light	7	268	-127	20.0	1	<0.0001	1.000	1.0
Fert + Light + Tube	8	269	-126	1.3	1	0.25	0.418	1.4

Table S5. Effect (pH measured in 2007) of experimental fertilization and supplementary understory light. Results are shown as mean and 95% CI.

2006	pH		
Source	Effect	2.5%	97.5%
Control	7.23		
Fertilization	*-0.07*	-0.15	0.02
Light	*0.02*	-0.07	0.11
Fertilization + Light	*-0.04*	-0.13	0.05

The effects are reported as the value for the control and the differences (in italics) between control and the other treatments.

Table S6. Species occurring for each treatment of the four different species mixtures (pool 1 - 4). In bold, species that were originally in the pre-established 6-species mixture pool. In parenthesis, the species that were lost or gained between 2006 and 2007 are represented by + and – respectively for each replicate. The species that were present in both years are represented by 0 (no change) and the species that were absent in both years from one of the replicates are represented by x. Nomenclature follows Flora Helvetica (*S9*).

Treatment	Species name			
	Pool 1	Pool 2	Pool 3	Pool 4
Control	**Arrhenatherum elatius (0,0)** Crepis biennis (+,x) Festuca pratensis (0,-) **Festuca rubra (-,0)** **Gallium mollugo (0,0)** Geranium pratense (x,0) Lolium perenne (-,-) Medicago lupulina (-,x) Plantago major (x,+) Poa pratensis (+,-) Poa Trivialis (x,+) Polygonum aviculare (x,-) Rumex acetosa (+,x) **Taraxacum officinale (-,-)** Trisetum flavescens (+,+) **Trifolium pratense (0,0)**	Alopecurus pratensis (+,x) Anagallis arvensis (+,x) **Centaurea jacea (0,0)** Dactylis glomerata (+,x) Festuca rubra (+,x) **Knautia arvensis (0,-)** Lolium perenne (x,+) Lychnis flos-cuculi (+,x) **Medicago lupulina (0,0)** Myosotis arvensis (0,-) **Phleum pratense (x,-)** **Plantago lanceolata (-,0)** **Poa pratensis (0,0)** Poa Trivialis (x,+) Silene nutans (x,+) Taraxacum officinale (0,0) Trisetum flavescens (+,0) Trifolium pratense (x,+) Veronica persica (x,0)	Achillea millefolium (-,x) Anagallis arvensis (x,-) Arrhenatherum elatius (x,+) Cerastium fontanum (x,+) Conyza canadensis (x,-) Festuca pratensis (x,-) **Holcus lanatus (0,x)** Lactuca serriola (x,-) Lamium purpureum (x,+) Lolium perenne (0,+) **Lychnis flos-cuculi (0,0)** Medicago lupulina (+,x) Poa pratensis (-,x) Taraxacum officinale (x,0) **Trisetum flavescens (0,0)** Trifolium pratense (x,0) **Trifolium repens (-,-)** Veronica persica (-,0)	**Achillea millefolium (-,-)** **Crepis biennis (-,-)** **Dactylis glomerata (0,0)** **Festuca pratensis (0,-)** Gallium mollugo (-,x) **Geranium pratense (0,0)** Lactuca serriola (x,-) Lathyrus pratensis (x,-) Lolium perenne (x,+) Medicago lupulina (-,x) Phleum pratense (x,-) Poa pratensis (+,-) Poa Trivialis (-,+) Setaria viridis (x,+) Taraxacum officinale (0,+) Trifolium repens (+,x) Veronica persica (x,0) **Vicia cracca (0,0)**
Fertilization	Achillea millefolium (-,x) **Arrhenatherum elatius (0,0)** Festuca pratensis (x,-) **Festuca rubra (+,0)** **Gallium mollugo (0,0)** Geranium pratense (x,0) Lolium perenne (x,-) Medicago lupulina (x,-) Poa pratensis (-,-) Poa Trivialis (+,x) Sonchus asper (x,-) **Taraxacum officinale (-,-)** Trisetum flavescens (0,+) **Trifolium pratense (0,0)** Veronica persica (-,-)	**Centaurea jacea (0,-)** Cerastium fontanum (-,x) Dactylis glomerata (-,x) Festuca pratensis (-,x) Gallium mollugo (x,-) **Knautia arvensis (-,-)** Lolium perenne (x,+) Lychnis flos-cuculi (-,-) **Medicago lupulina (0,0)** Myosotis arvensis (0,-) **Plantago lanceolata (x,-)** **Poa pratensis (0,0)** Poa Trivialis (+,+) Sonchus asper (x,+) Taraxacum officinale (0,0) Trifolium pratense (x,+) Veronica persica (x,0)	Alopecurus pratensis (x,+) Anagallis arvensis (+,x) Cerastium fontanum (x,-) Festuca pratensis (x,-) **Holcus lanatus (-,0)** Lamium purpureum (+,x) Lepidium campestre (+.x) Lolium perenne (+,0) **Lychnis flos-cuculi (0,0)** Papaver rhoeas (-,x) Phleum pratense (x,-) Poa pratensis (x,-) Rumex acetosa (0,x) **Silene nutans (+,x)** Taraxacum officinale (-,-) **Trisetum flavescens (-,0)** Trifolium pratense (x,0) **Trifolium repens (-,x)** Veronica persica (-,x)	**Achillea millefolium (-,-)** Arrhenatherum elatius (-,x) **Crepis biennis (-,-)** **Dactylis glomerata (0,0)** **Festuca pratensis (0,-)** **Geranium pratense (0,0)** Lactuca serriola (x,-) Lolium perenne (x,+) Poa pratensis (-,+) Poa Trivialis (+,-) Taraxacum officinale (0,-) Veronica persica (x,-) **Vicia cracca (0,0)**
Light	**Arrhenatherum elatius (0,0)** Festuca pratensis (0,x) Festuca rubra (0,+) **Gallium mollugo (0,0)** Geranium pratense (x,0) Knautia arvensis (+,x) Lolium perenne (-,x) Medicago lupulina (0,x) Plantago lanceolata (x,+) Poa pratensis (+,x) Poa Trivialis (x,+) Rumex acetosa (x,+) Silene nutans (+,x) **Taraxacum officinale (-,0)** Trisetum flavescens (+,+) **Trifolium pratense (0,0)** Veronica persica (0,-)	**Centaurea jacea (0,0)** Conyza canadensis (-,x) Dactylis glomerata (x,-) Festuca pratensis (x,+) Gallium mollugo (-,+) **Knautia arvensis (0,-)** Lolium perenne (x,+) Lychnis flos-cuculi (+,+) **Medicago lupulina (0,0)** Myosotis arvensis (0,x) **Phleum pratense (-,x)** **Plantago lanceolata (+,+)** Plantago major (+,x) **Poa pratensis (0,0)** Poa Trivialis (+,+) Setaria viridis (x,+) Taraxacum officinale (0,0) Trisetum flavescens (x,0) Trifolium pratense (x,0) Veronica persica (-,0)	Anagallis arvensis (-,-) Arrhenatherum elatius (x,+) Cerastium fontanum (+,x) Cirsium arvense (-,x) Conyza canadensis (-,-) Festuca pratensis (-,x) Gallium mollugo (+,x) **Holcus lanatus (x,0)** Lamium purpureum (-,+) Lolium perenne (+,+) **Lychnis flos-cuculi (0,0)** Medicago lupulina (-,+) Papaver rhoeas (-,x) Phleum pratense (x,-) Plantago lanceolata (+,x) Plantago major (x,+) Poa pratensis (-,-) Rumex acetosa (+,x) Setaria viridis (x,+) Sonchus asper (-,x) Taraxacum officinale (-,+) **Tragopogon pratensis (-,x)** **Trisetum flavescens (-,0)** Trifolium pratense (-,0) **Trifolium repens (x,-)** Veronica persica (0,0) Viola arvensis (-,x)	**Achillea millefolium (-,-)** Anagallis arvensis (x,+) Cerastium fontanum (-,x) **Crepis biennis (-,-)** **Dactylis glomerata (0,0)** **Festuca pratensis (0,-)** **Geranium pratense (0,0)** Lactuca serriola (x,-) Lamium purpureum (x,-) Lolium perenne (x,+) Medicago lupulina (0,-) Poa pratensis (-,+) Poa Trivialis (+,-) Silene nutans (x,+) Taraxacum officinale (0,0) Veronica persica (x,0) **Vicia cracca (0,0)**
Fertilization + Light	Achillea millefolium (+,x) **Arrhenatherum elatius (0,0)** Festuca pratensis (x,-) **Festuca rubra (-,-)** **Gallium mollugo (0,0)** Geranium pratense (x,0) **Leucanthemum vulgare (-,x)** Lolium perenne (0,-) Medicago lupulina (-,x) Poa pratensis (0,x) Poa Trivialis (x,+) **Taraxacum officinale (-,0)** Trisetum flavescens (+,+) **Trifolium pratense (0,0)** Veronica persica (-,0)	**Centaurea jacea (0,0)** Conyza canadensis (x,+) Dactylis glomerata (0,x) Festuca pratensis (x,0) Festuca rubra (x,+) Holcus lanatus (x,+) **Knautia arvensis (-,-)** Lolium perenne (x,0) Lychnis flos-cuculi (+,-) **Medicago lupulina (0,0)** Myosotis arvensis (0,x) **Phleum pratense (-,-)** **Plantago lanceolata (0,+)** **Poa pratensis (0,0)** Poa Trivialis (x,+) Silene nutans (+,x) Taraxacum officinale (0,0) Trisetum flavescens (0,x) Veronica persica (-,-)	Alopecurus pratensis (+,+) Anagallis arvensis (-,-) Arrhenatherum elatius (+,0) Conyza canadensis (-,x) Festuca pratensis (0,+) **Holcus lanatus (0,0)** Lamium purpureum (0,x) Lolium perenne (0,0) **Lychnis flos-cuculi (0,0)** Phleum pratense (x,-) Plantago lanceolata (+,x) Poa pratensis (-,-) Polygonum aviculare (x,-) Rumex acetosella (-,x) Setaria viridis (x,+) **Silene nutans (x,+)** Taraxacum officinale (-,+) **Trisetum flavescens (0,0)** Trifolium pratense (x,0) Veronica persica (0,0)	**Achillea millefolium (0,-)** **Crepis biennis (-,-)** **Dactylis glomerata (0,0)** **Festuca pratensis (-,-)** Gallium mollugo (+,x) **Geranium pratense (0,0)** Knautia arvensis (+,x) Lolium perenne (0,0) Medicago lupulina (0,x) Poa pratensis (0,-) Poa Trivialis (+,+) Setaria viridis (+,x) Taraxacum officinale (0,0) Veronica persica (x,0) **Vicia cracca (0,0)**

Table S7. Linear model results of the response of temperature to the distance from the lamp (cm) and the two levels of understory light treatment in the experimental glasshouse at University of Zurich in 2007. The intercept is the temperature of the lamp in the closed light treatment. Enclosed lights increased temperature by the same amount as open lights. Results are shown as mean and 95% CI.

	Temperature		
Source	Effect	2.5%	97.5%
Intercept	23.7		
Distance	-0.1	-0.2	-0.1
Light	0.1	-0.3	0.4
Distance*Light	0.1	-0.1	0.1

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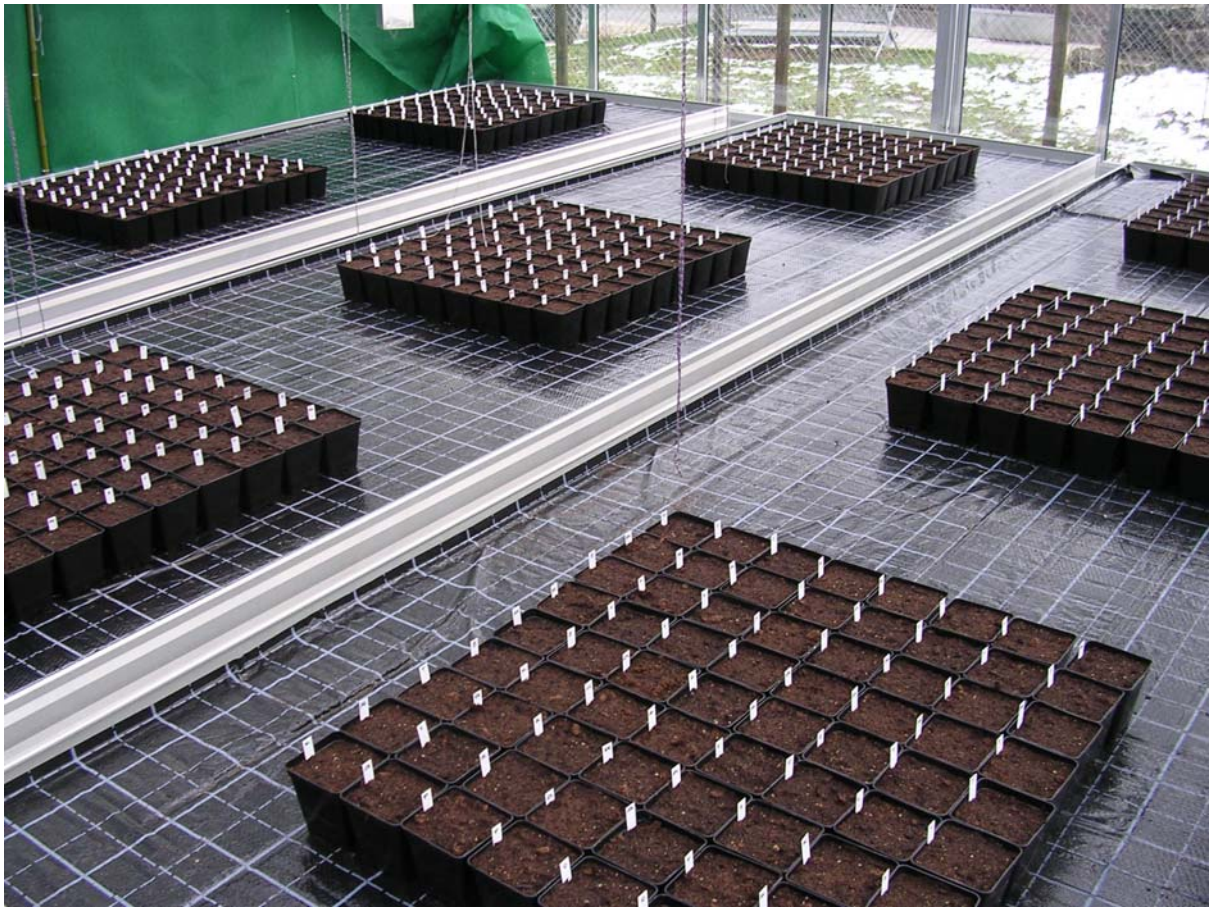
Chapter 3

Modelling the growth of parasitic plants

Yann Hautier, Andy Hector, Eva Vojtech, Drew Purves & Lindsay A. Turnbull (*Journal of Ecology* in review)



Abstract

1. Hemiparasitic plants, such as *Rhinanthus* species, have substantial effects on community composition and biomass. For example, the presence of parasites often increases diversity but reduces the combined biomass of hosts and parasites by roughly 25% compared with unparasitised controls. We present and test a simple model of the host-parasite interaction in which parasite growth rate is a function of host growth rate that offers a new explanation for why hemiparasitic plants reduce ecosystem productivity.
2. The model predicts that the combined mass of the host-parasite system is always less than the mass of the host grown alone because the combined biomass is dependent only on host growth rate, which is reduced by the parasite. The model also predicts that the parasite should adopt an intermediate virulence to maximise its own performance, but that the optimum virulence depends on host growth characteristics.
3. The key assumption of the model is that parasite growth rate and hence parasite biomass is tightly coupled to host growth rate. We tested this assumption by measuring the performance of *Rhinanthus alectorolophus*, a widespread hemiparasitic annual plant, on nine common European grass species. First, we determined size-corrected growth rates for the grasses by fitting power-law growth curves to multiple-harvest data on host individuals grown without *Rhinanthus*. Second, we grew *Rhinanthus* on each of the grass species and related *Rhinanthus* final biomass to the grass species' growth rates.
4. *Rhinanthus* performance was strongly correlated with the growth rate of the host grass species, thus validating a key assumption of our model. However, *Rhinanthus* biomass on three of the nine grass species differed significantly from the value predicted based on host growth rate alone, suggesting that grass species differ in their resistance to parasitism.
5. *Synthesis.* Parameterising such models of the host-parasite relationship could help to explain variation in *Rhinanthu*s performance on different hosts; variation in the effects of hemiparasites in grasslands of different productivity; and differences in virulence among parasite populations.

Keywords: Coexistence, grassland restoration, host-parasite interaction, hemiparasitic plants, perennial grass species, relative growth rate (RGR), *Rhinanthus*, virulence.

Introduction

Parasitic plants are one of the largest, most ubiquitous and diverse plant groups, represented by more than 3000 species worldwide and occurring in many different habitats (Press & Graves 1995; Press 1998). In Europe, the most common species are root hemiparasites of the family Orobanchaceae, e.g. *Rhinanthus* species, which occur widely in natural and semi-natural grasslands (Gibson & Watkinson 1989; Matthies & Egli 1999; Joshi *et al.* 2000). Such hemiparasites use a wide range of hosts, but their presence has a profound effect on species composition, suggesting unequal effects on host species (Gibson & Watkinson 1991; Davies *et al.* 1997; Pywell *et al.* 2004; Bardgett *et al.* 2006; Bullock *et al.* 2007; Pywell *et al.* 2007; Grewell 2008; Niemela *et al.* 2008).

Rhinanthus performance also differs among host species (Matthies 1996) and some hosts are known to be resistant (Cameron *et al.* 2006). Hemiparasites can have dramatic effects on the functioning of ecosystems, affecting standing crop, nutrient cycling, decomposition rate and interactions with other trophic levels such as herbivores and pollinators (Marvier 1996; Joshi *et al.* 2000; Adler 2002; Phoenix & Press 2005; Press & Phoenix 2005; Quested *et al.* 2005; Bardgett *et al.* 2006; Ameloot *et al.* 2008). In this paper we present and test a simple model of the host-parasite relationship which helps to explain both individual and system-level effects of hemiparasites.

In contrast to holoparasites, hemiparasites are photosynthetically active, but they rely on the host for water and mineral nutrients which they extract through specialised structures called haustoria (Seel & Press 1993; Press & Graves 1995; Seel & Jeschke 1999). These structures allow the parasite to access the xylem vessels of the host; for example, Jiang et al. (2004) estimated that *Rhinanthus minor* withdraws ca. 18% of host nitrogen, 22% of host phosphorus and 20% of host potassium when attached to barley (*Hordeum vulgare*). The removal of nutrients from the host by a hemiparasite clearly compromises host performance (Matthies 1995) and this, coupled with the high densities that hemiparasites can achieve in the field (Westbury 2004), can have dramatic effects on standing crop. A meta-analysis reporting the effect of *Rhinanthus* species (Ameloot *et al.* 2005) showed that the aboveground biomass of host species is reduced by ~ 50 % in the presence of *Rhinanthus* compared with unparasitised control plots, and that the total combined biomass of hosts and parasites is reduced by ~25%. The reduction in the combined biomass of host and parasite compared with unparasitised host biomass has been attributed to reduced nitrogen-use efficiency by the parasite compared with the host (Matthies 1995; Ameloot *et al.* 2005) or to reductions in host photosynthesis (Watling & Press 2001; Cameron *et al.* 2008). Here we use a simple model to propose an alternative explanation. If only host plants (but not the parasite) take up resources which limit system productivity, then extraction of resources from the host by the parasite, leads to reduced future resource extraction and hence reduced system productivity. In this respect our model differs from other attempts to model host-parasite interactions which do not generally consider the growth of individuals (Smith *et al.* 2003; Cameron *et al.* 2009).

Our simple model also illustrates how observed differences in parasite performance can be due to differences in host growth rate (faster-growing host species may simply provide more resources to the parasite), or to differences in host resistance (host species may differ in the ease with which *Rhinanthus* can both form and maintain connections (Cameron *et al.* 2006; Rumer *et al.* 2007). Some host species are known to be resistant; for example, *Plantago lanceolata* can encapsulate the parasite's invading structures (Cameron *et al.* 2006; Rumer *et al.* 2007) and prevent the removal of host nitrogen (Cameron & Seel 2007); however, grass species are generally considered to be good hosts (Gibson & Watkinson 1991). Nevertheless, variation in *Rhinanthus* performance among grass species has been reported (Gibson & Watkinson 1991; Niemela *et al.* 2008) which could be due to variation in grass growth rates (Grime & Hunt 1975) or to variation in resistance among grasses (grass roots have been observed to lignify root cells in response to Rhinanthus haustoria, indicating some potential for resistance: Rumer *et al.* 2007).

We present a simple model for a host-parasite interaction in which parasite growth rate is a simple function of host growth rate. We use the model to demonstrate that 1) the host-parasite system always exhibits reduced combined yield compared

with a host growing alone; 2) all else being equal, a faster growing host species will result in a greater final biomass of the parasite; 3) in most cases an intermediate level of host resource extraction optimises parasite performance; 4) the optimum level of host resource extraction depends on the type of growth experienced by host plants. We tested the model assumptions by conducting an experiment in which we first determined growth rates for nine host grass species by fitting growth curves to multiple-harvest data on host individuals grown alone (without the parasite). We then measured the performance of the hemiparasitic plant *Rhinanthus alectorolophus* from different sub-populations and maternal genotypes on the same nine host species and related parasite performance to host growth rates.

Material and methods

A simple model for *Rhinanthus* growth

To understand how the growth of the host plant and the parasite might be coupled, it is easiest to start with the very simple case of the growth of a single parasitic plant (e.g. a single *Rhinanthus*) attached to a single host. Although there are a large number of potential formulations for plant growth (Hunt 1982), we chose the power-law growth equation advocated by West *et al.* (1997). In this formulation, the instantaneous change in host biomass (M_H) per unit time (t) when growing without the parasite is:

$$\frac{dM_H}{dt} = \beta_H M_H{}^{\alpha} \qquad \text{Eqn 1}$$

where β_H is a growth coefficient and α is the scaling exponent. This has the following analytical solution:

$$M_{H,t} = \left(M_{H,0}^{1-\alpha} + \beta(1-\alpha)t\right)^{1/(1-\alpha)} \qquad \text{Eqn 2}$$

where $M_{H,0}$ is the initial mass of the host and t is the time in days after germination (see also Muller-Landau *et al.* 2006; Russo *et al.* 2007). Notice that we have made no explicit assumption about aboveground vs. belowground limitation; we have only assumed that the absolute host growth rate (increase in mass per unit time) is mass-dependent, i.e., that it increases with the biomass of the host plant. We now assume that parasite growth is totally dependent on host growth. This is presumably always true for holoparasites, but is nearly true for hemiparasites such as *Rhinanthus*, which can barely grow when unattached under nutrient-poor conditions (Matthies 1995). We assume simply that the parasite receives a constant fraction F_R of the host growth:

$$\frac{dM_R}{dt} = F_R \beta_H M_H{}^{\alpha} \qquad \text{Eqn 3}$$

The removal of resources by the parasite causes a reduction in the host growth rate, which is now given by:

$$\frac{dM_H}{dt} = (1-F_R)\beta_H M_H{}^{\alpha} \qquad \text{Eqn 4}$$

These equations can be solved, giving for the host:

$$M_{H,t} = \left(M_{H,0}^{1-\alpha} + [1-F_R]\beta_H(1-\alpha)t\right)^{1/(1-\alpha)} \qquad \text{Eqn 5}$$

and for the parasite (ignoring the initial mass of the parasite):

$$M_{R,t} = \frac{F_R}{[1-F_R]}\left(M_{H,t} - M_{H,0}\right) \qquad \text{Eqn 6}$$

Inspection of Eqn 5 shows that the final host biomass is higher for: 1) high values of the host growth rate parameter β_H; 2) high values of the scaling exponent α; and 3) a lower degree of parasitism (i.e. lower F_R). The final biomass of the parasite is also higher when attached to a fast-growing host (with a high value of β_H and/or a high value of α). How-

ever, the final biomass of the parasite has a more complex relationship with the degree of parasitism, with intermediate values of F_R giving higher final parasite biomass (see below).

The model is highly simplified, for example, the fraction of resources removed by the parasite is unlikely to be constant, but we believe that this simple model is sufficient to capture essential features of the system. We used eqns 5 and 6 to investigate the effect of parasitism on the mass of the host, the mass of the parasite and their combined mass for different values of F and α over the typical 90-day growth period of a hemiparasite such as *Rhinanthus* assuming biologically realistic parameters ($M_{H,0} = 1$ g and $\beta_H = 0.2$).

Estimating growth rates of host grass species.

The key assumption of the model is that parasite growth rate, and hence parasite final biomass, is tightly coupled to host growth rate. To test this assumption we selected nine common European perennial grass species as potential hosts for *Rhinanthus*: *Agrostis capillaris*, *Alopecurus pratensis*, *Anthoxanthum odoratum*, *Arrhenatherum elatius*, *Bromus erectus*, *Dactylis glomerata*, *Festuca rubra*, *Holcus lanatus* and *Trisetum flavescens* (Lauber & Wagner 2001). Grasses are generally not considered to be resistant to *Rhinanthus*, but they vary greatly in their growth rates, hence we expect highly variable parasite performance due to growth rate differences among grasses. Each grass species was grown under each of three different light regimes (see below) and Equation 2 was fitted to the resulting repeated harvest data allowing estimates of α_i and β_i. To provide a simple comparison of species growth rates unbiased by differences in seed sizes (Turnbull *et al.* 2008; Table 1) we used the parameterized eqn. 2 to calculate an absolute growth rate at a common reference size, M_c. Similarly, the size-corrected RGR (Enquist *et al.* 1999) is then given by

$$RGR = \frac{1}{M_c}\frac{dM_i}{dt} = \beta_i M_c^{(\alpha_i - 1)} \qquad \text{Eqn 7}$$

If – as suggested by West *et al.* (1997) – species share a common value of α_i , then differences among species in growth rate at any given common mass are encapsulated by a single parameter, β_i , and the relative ranking of species will be independent of the choice of common size (M_c). If, however, species require different values of α_i then the relative rankings of species may change depending on the common size chosen. If both AGR and RGR are size-corrected, either can be used to make unbiased comparisons among species (if $M_i = M_c$ then RGR is simply AGR divided by a constant: see Eqn. 7).

Data Collection

From April 2006 individual grass plants were grown from seed for 97 days in a glasshouse in 0.6 l pots containing a mixture of 1:1 peat and sand. For each grass species, eight pots were randomly assigned to three light treatments (9 species x 8 harvests x 3 light levels x 3 replicates = 648 plants). Light was manipulated using shade cloths giving three light levels: control (no shade cloth, 100% daylight), 42% daylight and 11% daylight. Seeds of different species were germinated synchronously and were harvested nine times on days 7, 14, 21, 28, 42, 56, 70, 83 and 97 following germination. Individuals that did not survive were discarded meaning that the number of plants per harvest and per species was between 1 and 3 giving a final total of 629 plants harvested. Plants were irrigated automatically on a daily basis. We measured aboveground plant biomass by clipping the plants at soil level, drying at 80 ºC for 48 hours and weighing. The power-law relationship described in equation

Table 1. Seed weight of 100 seeds of the nine experimental grass species.

Plant name	Seed weight of 100 seeds in g
Ag: Agrostis capillaris	0.027
Al: Alopecurus pratesis	0.089
An: Anthoxanthum odoratum	0.053
Ar: Arrhenatherum elatius	0.275
B: Bromus erectus	0.539
D: Dactylis glomerata	0.0102
F: Festuca rubra	0.089
H: Holcus lanatus	0.045
T: Trisetum flavescens	0.028

2 can be difficult to fit because of convergence problems if the nitial condition ($M_{H,0}$; Eqn 2) is included as a free parameter in addition to α_i and β_i. To get convergence, we decided to fix (rather than estimate) the initial biomass of each species on day 0, i.e. germination day, since this can be measured with considerable accuracy. We measured initial seedling size on the day of germination by placing 10 seeds of each grass species on filter paper in a Petri dish. On the day of emergence the shoot was removed, dried and weighed.

Model fitting

We fitted the model (Eqn 2) using generalized nonlinear least squares with the *gnls* function from the *nlme* library (Pinheiro & Bates 2000) for R 2.8.1. (R Development Core Team 2008). Light availability (% daylight) was log-transformed and fitted as a continuous variable. Because biomass is not log-transformed, initial residuals were strongly heteroscedastic but could be modelled as a power function of time (using the function *varPower*). We used a model-building approach, in which we compared models of increasing complexity, considering models in which both α_i and β_i were functions of light availability and species identity. We identified the most parsimonious model based on minimization of AIC. There was relatively weak evidence for species differences in α_i compared to β_i (see *Results*). Thus, to compare the influence of increasing model complexity on estimated grass growth rates we calculated size-corrected AGR (Eqn 4) using parameters taken from two different models: 1) AGR_α calculated from a model in which the scaling exponent, α was light-dependent but common across all species; and 2) AGR_{α_i} calculated from a model in which the scaling exponent, α_i was both light-dependent and species-specific. For the model in which α_i was species-specific, relative rankings of species can change depending on the common mass selected and the light level. We investigated this effect by calculating the dependency of AGR_{α_i} on mass and light level within the experimental range.

Performance of *Rhinanthus alectorolophus* on different host grass species

Data collection

In June 2006, we collected seeds from four individuals of *Rhinanthus alectorolophus* (*Rhinanthus*) at four sites in semi-natural grasslands around the University of Zurich, giving 16 maternal genotypes in total. The sites were selected because they appeared to differ in the composition and abundance of host species, although this was not quanti

fied. In September 2006, 0.6 l pots filled with the same peat/sand mixture as used for the grass growth experiment were sown with 20 seeds of a single grass species and placed outside in the experimental garden of the University of Zurich (47° 23' N, 8° 33' E, and 546 m height a.s.l.). Each of the four maternal genotypes of *Rhinanthus* from each of the four sites was grown on each of the nine host species by sowing nine seeds from a single maternal genotype into each pot at the same time as the grass seed was sown (4 sites x 4 maternal genotypes x 9 grass species). In addition, each maternal genotype was grown without a host (4 sites x 4 maternal genotypes) and each grass species was grown without *Rhinanthus* (9 species x 2 replicates) for a total of 178 pots. The grass seeds germinated quickly and grew during the mild autumn to form relatively large plants by the following spring. The *Rhinanthus* seeds germinated quite synchronously at the beginning of March and at this time we estimated the percentage cover of grass in each pot and harvested all grass hosts growing without *Rhinanthus*. We used the relationship between percentage cover, host species and grass biomass in harvested pots to estimated initial host biomass in the remaining unharvested pots. At the beginning of May 2007, approximately eight weeks after germination of *Rhinanthus* seeds, pots were harvested by clipping all plants at soil level, counting the number of *Rhinanthus* individuals present, drying at 80 ºC for 48 hours and weighing the biomass of both host and parasite.

Statistical analysis

We first analyzed the performance of *Rhinanthus* (final biomass) with linear mixed-effects models (Pinheiro & Bates 2000) using the *lme* function from the *nlme* library for R 2.8.1. The number of *Rhinanthus* individuals and host AGR (Eqn 1) were fitted as continuous variables and treated as fixed effects. *Rhinanthus* population (site of provenance) and *Rhinanthus* maternal genotype were treated as nested random effects. As the variation explained by the random terms was not significant (see *Results*) the random effects were dropped and we were able to use ordinary least-squares regression. We calculated the AGR of host plant species in four ways using parameters taken from different models under 100% light level and also by assuming either a common initial mass for all species or by incorporating differences in initial mass estimated for each pot (see *Methods: Data collection*). Specifically, using equation 1, we calculated for each host species: 1) $AGR_{\alpha M}$ estimated from a model with species-specific values of β_i and a single common value of α calculated at a single common size (the estimated average mass of plants in the glasshouse); 2) $AGR_{\alpha_i M}$ estimated from a model with species-specific values of both α_i and β_i calculated at a single common size (the estimated average mass of plants in the glasshouse); 3) $AGR_{\alpha M_i}$ estimated from a model with species-specific values of β_i and a single value of α and calculated at a unique mass for each host (the estimated initial host mass for each pot in the garden); 4) $AGR_{\alpha_i M_i}$ estimated from a model with species-specific values of both α_i and β_i and calculated at a unique mass for each host (the estimated initial host mass for each pot in the garden). We identified the most parsimonious set of parameters by comparing the fit of models based on minimization of AIC.

Results

Model behaviour

As long as the host growth rate is mass dependent, the parasite always reduces the total biomass of the system because it reduces the instantaneous host growth rate, an effect that compounds with time. To understand why, it helps to begin with the specific case in which

host growth rate is *not* mass-limited (i.e. α = 0). In this special case, the combined growth rate of the host and parasite is constant, depending only on the host growth parameter β_i. Therefore, the combined final mass of the host and parasite is insensitive to the degree of parasitism (F_R). But, the fraction of this final biomass that is assigned to the parasite, rather than the host, is set by F_R (Fig 1A). In this special case, the best solution for the parasite is to extract the maximum possible resources from the host, as resource extraction does not compromise future host growth.

However, mass-independent growth throughout the whole life cycle of a plant is unlikely. Rather, plants usually exhibit an exponential-like phase of growth, during which growth rate increases as mass increases. This is captured by our scaling exponent, α. As α increases, host growth is increasingly mass-dependent, such that the reduction in host biomass caused by the resource extraction of the parasite reduces future host growth. In this case, very high resource extraction by the parasite reduces host growth so much that final parasite biomass is strongly compromised. However, very low resource extraction also leads to low final parasite biomass, simply because the parasite has taken so few resources from the host. Hence, the optimum resource extraction level by the parasite is intermediate (Fig 1B). As the value of α increases – that is, as host biomass becomes increasingly mass-limited – the effect of resource extraction by the parasite on host mass and the combined mass of the system becomes more dramatic, and parasite performance is more severely

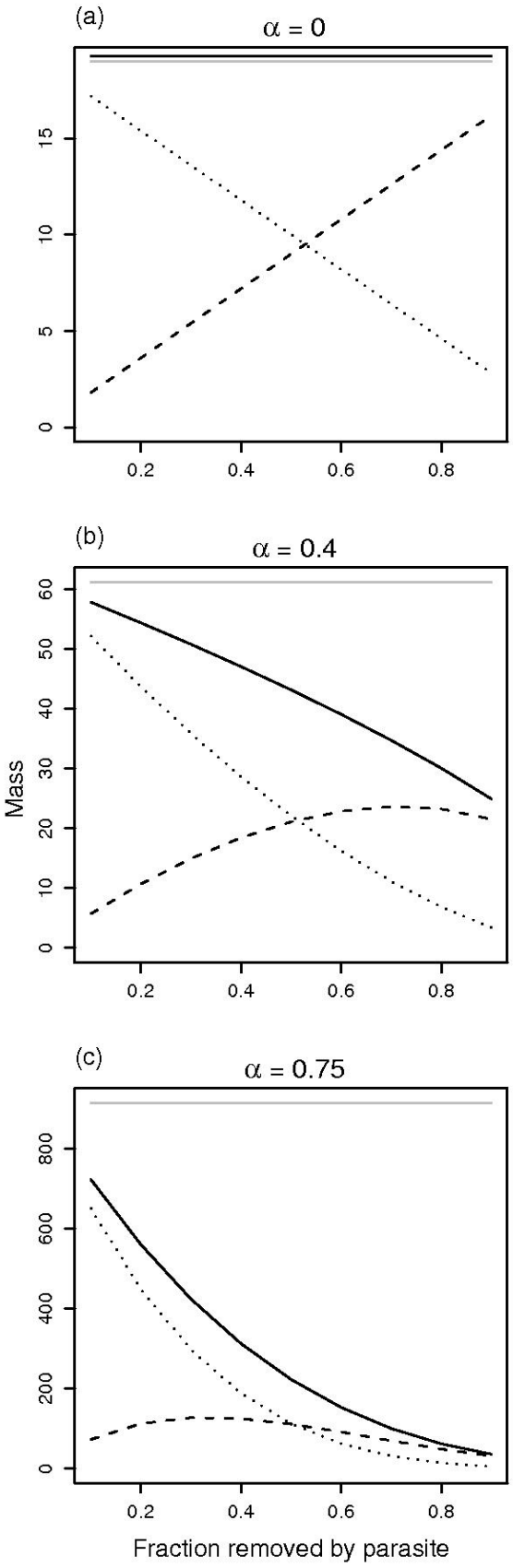


Figure 1: Results of a host-parasite model in which the host grows according to a power-law with a scaling exponent (α) and the parasite removes a constant fraction (F_R) of the host growth rate. The host mass when grown alone (grey, solid), the host mass when grow with the parasite (dotted) and the parasite mass (dashed), together with their combined mass (black solid) are shown. Note different range of y axis in panel a-c. In (a) the grey line has been shifted down for visibility.

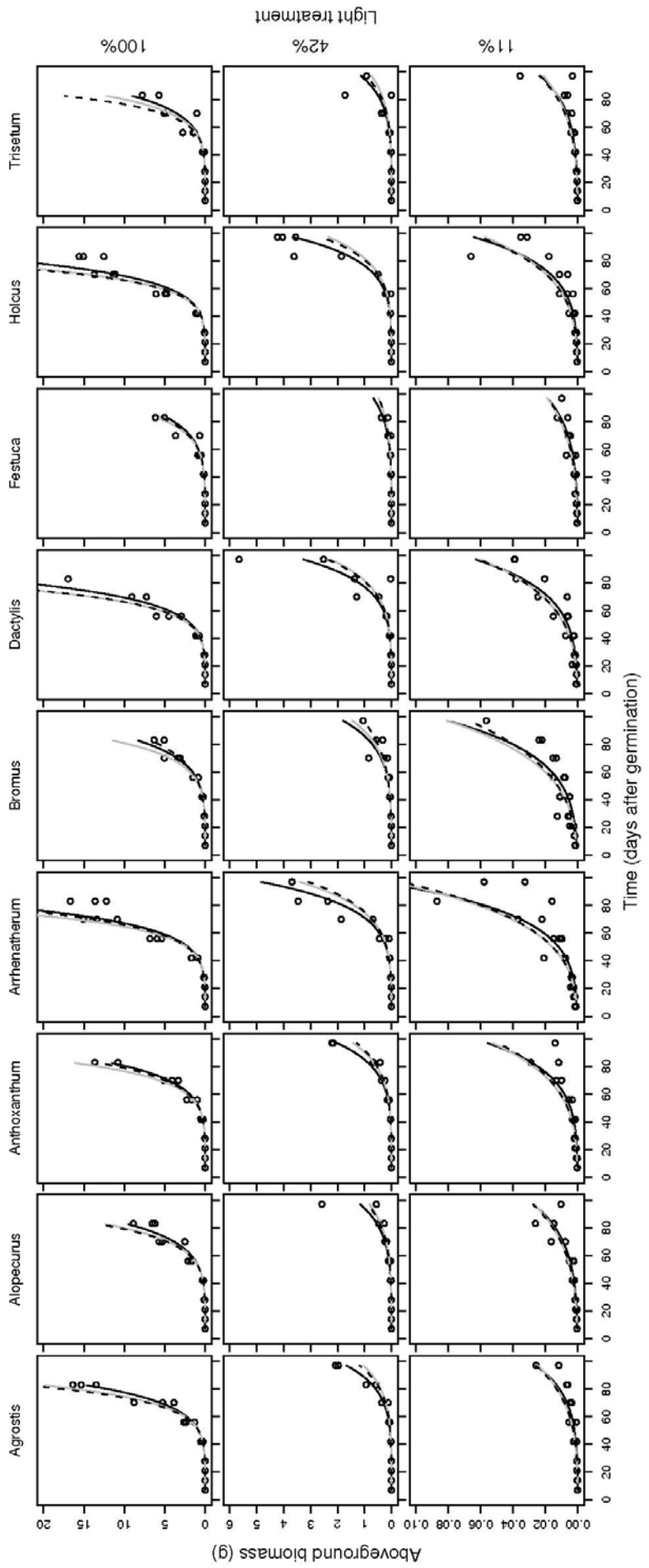


Figure 2: Fitted growth rate curves for nine common perennial grass species grown under three light levels (upper panel: 100% daylight; middle panel: 42% daylight; and lower panel: 11% daylight). The growth rates are from fitted models with a single common value of α (black, solid), with α light-dependent but not species-specific (grey, solid) or with α both light-dependent and species-specific (dashed). Note decreasing range of biomass (y axis) going from the top to bottom row.

Table 2. Average biomass M and parameter estimates (α and β; eqn 1) from different growth models. $\beta_{i,\alpha}$ are the values estimated from a model with a single common value of α among species and light treatments. β_{i,α_l} are the values estimated from a model where α_l is light-dependent but not species-specific. β_{i,α_i} are the values estimated from a model where α_i is both light-dependent and species-specific. M is the estimated average mass of plants in the glass-house.

Light treatment	Species	$\beta_{i,\alpha}$	β_{i,α_l}	β_{i,α_i}	α	α_l	α_i	M (g)
	Agrostis capillaris	0.106	0.116	0.120			0.887	
	Alopecurus pratensis	0.093	0.101	0.103			0.886	
	Anthoxanthum odoratum	0.099	0.110	0.103			0.864	
	Arrhenatherum elatius	0.113	0.122	0.116			0.861	
100%	*Bromus erectus*	0.088	0.096	0.079		0.879	0.825	
	Dactylis glomerata	0.112	0.122	0.122			0.880	
	Festuca nigrescens	0.083	0.092	0.089			0.872	
	Holcus lanatus	0.116	0.127	0.129			0.886	
	Trisetum flavescens	0.095	0.105	0.116			0.904	
	Agrostis capillaris	0.062	0.053	0.056			0.849	
	Alopecurus pratensis	0.053	0.046	0.049			0.847	
	Anthoxanthum odoratum	0.062	0.054	0.051			0.825	
	Arrhenatherum elatius	0.068	0.061	0.058			0.823	
42%	*Bromus erectus*	0.055	0.050	0.040	0.857	0.837	0.786	2.325
	Dactylis glomerata	0.065	0.057	0.059			0.841	
	Festuca nigrescens	0.049	0.043	0.042			0.834	
	Holcus lanatus	0.069	0,060	0,063			0.847	
	Trisetum flavescens	0.056	0.047	0.056			0.865	
	Agrostis capillaris	0.027	0.015	0.018			0.789	
	Alopecurus pratensis	0.023	0.014	0.015			0.788	
	Anthoxanthum odoratum	0.030	0.018	0.017			0.766	
	Arrhenatherum elatius	0.031	0.021	0.020			0.763	
11%	*Bromus erectus*	0.027	0.018	0.014		0.772	0.726	
	Dactylis glomerata	0.028	0.018	0.019			0.781	
	Festuca nigrescens	0.022	0.013	0.013			0.774	
	Holcus lanatus	0.031	0.019	0.021			0.788	
	Trisetum flavescens	0.024	0.014	0.018			0.806	

compromised at high extraction rates. With our chosen parameters ($M_{H,0} = 1$ and β_H = 0.2), the optimum fraction of host resources to remove when α = 0.4 is $F_R \sim 0.7$, while when α = 0.75, the optimum fraction of host resources to remove is only $F_R \sim 0.3$ (Fig 1B & 1C).

Estimating growth rates of host grass species.

There was strong evidence that both the allometric constant β and the scaling exponent α were light-dependent ($F_{1,609}$ = 49536.2, P < 0.001 and $F_{1,609}$ =44.2, P < 0.001, for β and α

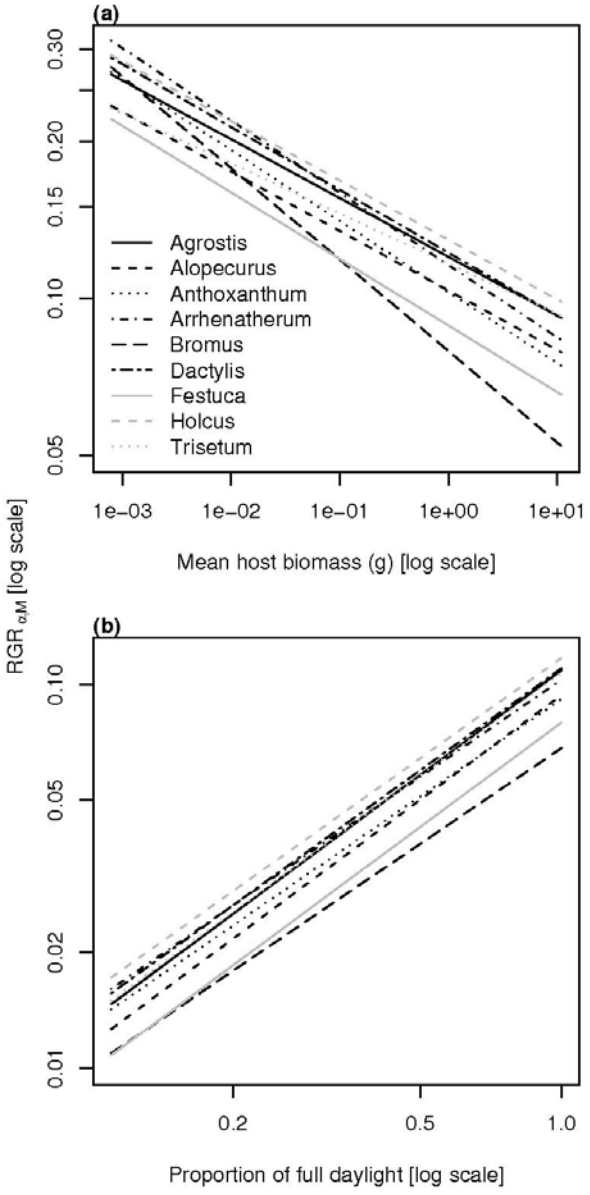


Figure 3: Relationship between the size-corrected relative growth rate (RGR) of the host when grown without the parasite, and (a) host biomass, (b) light availability. RGR was estimated at a common size (eqn 7) using parameters from models in which α_i and β_i are light-dependent and species-specific. For a) the common size (mean host biomass) ranges from the average initial mass of plants in the glasshouse to the average final mass of plants in the glasshouse. For b) RGR was calculated at a single common size (the average mass of plants in the glasshouse).

respectively) and varied among host species ($F_{8,609}$ =1063.1, $P < 0.001$ and $F_{8,609}$ =5.6, $P < 0.001$, for β and α respectively). However, although the best fit was obtained for the model in which α was both light-dependent and species-specific, the additional complexity made only limited changes to the fitted curves compared, for example, with models in which α varied with light-level but not with species (Fig. 2). When not allowed to vary among species and light treatments, the best estimate of the scaling exponent is α = 0.857 (s.e.m. = 0.004). When light-dependent, the value of α increases with increasing light availability (slope = 0.048); thus giving under 11% light, α = 0.833, under 42% light, α = 0.861 and under 100% light, α = 0.879. When light-dependent and species-specific, α_i varied in the range 0.782−0.904 (Table 2).

When using parameters from the most complex model (in which both α_i and β_i are species-specific) there were changes in the rankings of species' growth rates with both mass (M_c) and light (Fig. 3). Species' rankings can change with mass because the value of α_i determines how quickly growth slows with increasing mass; for example, *Bromus erectus* has the lowest value of α_i in our data set and therefore its growth rate declines more rapidly with size when compared with other species (Fig. 3a). Similarly, there is an imperfect correlation between species growth rates in different light levels (Fig. 3b). However, the change in rankings of species' growth rates was rather limited and there was certainly no evidence that species with the highest growth rates in low light have the lowest growth rates in high light (Sack & Grubb 2001; Kitajima & Bolker 2003).

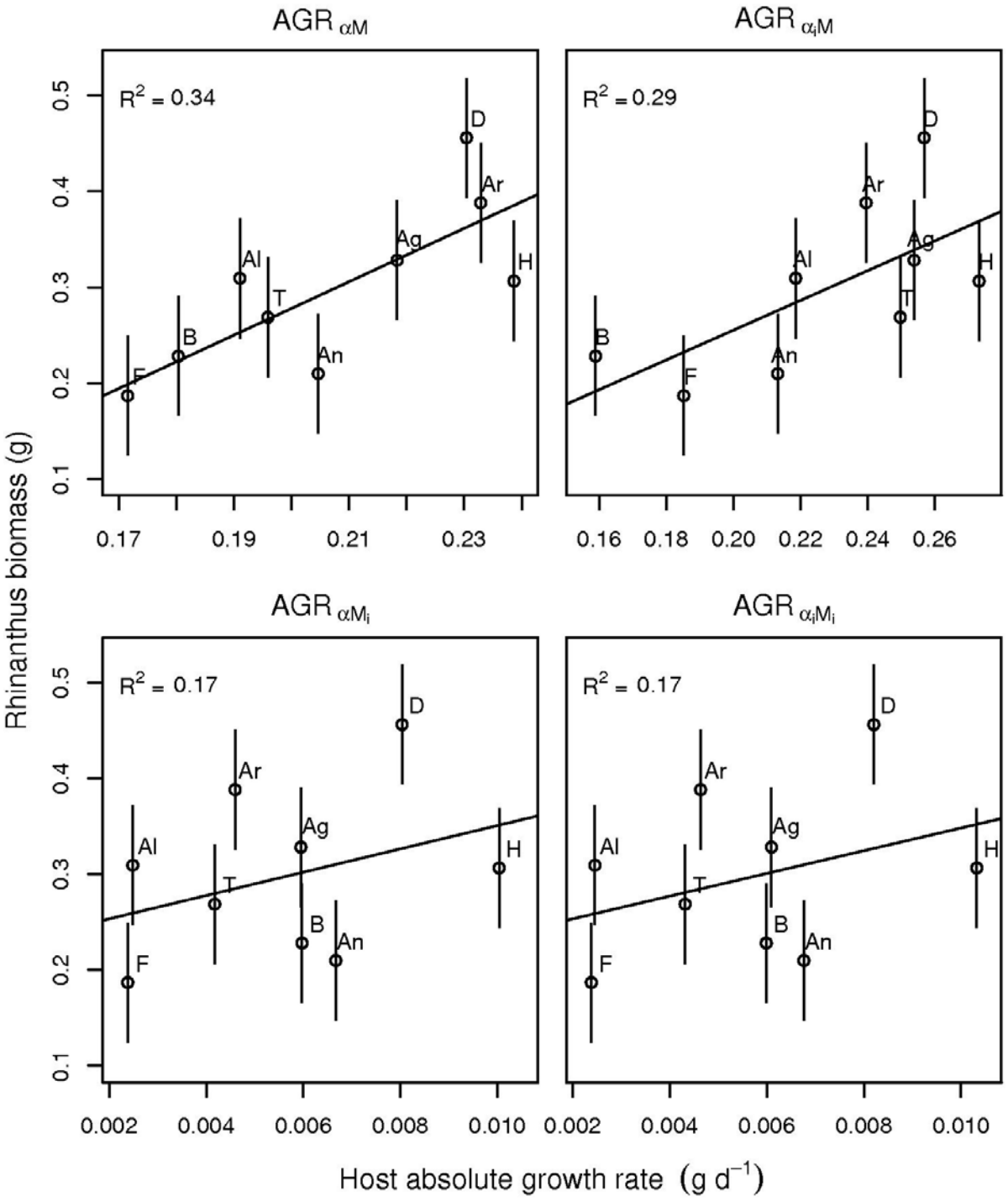


Figure 4: Relationship between the final biomass of *Rhinanthus* and the absolute growth rate (AGR) of nine common perennial host grass species (grown without the parasite). The different values of AGR are calculated using models of increasing complexity, see text for details of AGR calculations. Ag = *Agrostis capillaris*, Al = *Alopecurus pratensis*, An = *Anthoxanthum odoratum*, Ar = *Arrhenatherum elatius*, B = *Bromus erectus*, D = *Dactylis glomerata*, F = *Festuca rubra* ssp. *commutata*, H = *Holcus lanatus* and T = *Trisetum flavescens*. Means and 95% confidence intervals are shown. Note that the scale of the host absolute growth rate (x axis) is smaller on the two lowest panels because the estimated initial host mass in the garden was much smaller than the average mass in the glasshouse.

Performance of *Rhinanthus alectorolophus* on different host grass species

Rhinanthus individuals grown without hosts had an average biomass of only 0.037g (sd = 0.014) compared with an average mass of 0.297g (sd = 0.145) when grown with a grasshost (an ~8−fold difference), suggesting that under these conditions, *Rhinanthus* growth is strongly dependent on host growth. Of the four different estimates of host AGR, the best predictor of *Rhinanthus* biomass was that calculated using the simplest model, with a common scaling exponent α shared among species and where species are assumed to have the same initial mass (AIC = -191.4; $AGR_{\alpha M}$, Fig. 4a). Absolute growth rate calculated in this way was a substantially better predictor than any of the three more complex alternatives ($AGR_{\alpha_i M}$ AIC = -180.1, $AGR_{\alpha M_i}$ AIC = -159.9, and $AGR_{\alpha_i M_i}$ AIC = -160.0, Fig.4). Using the calculation of host AGR from the simplest model, differences in host growth rate are determined only by the species-specific allometric constant β_i. A substantial part of the variation in the performance of *Rhinanthus* was explained by this positive linear effect of grass growth rate (slope with 95% CI = 2.8 (1.8– 3.8), R^2 = 0.34; Fig. 3). There was no significant variation around this slope between the provenance of *Rhinanthus* mother plants (likelihood ratio test: χ^2 = 1.1, P = 0.29) or across *Rhinanthus* maternal genotypes (χ^2 = 0.6, P = 0.48). When using the best model ($AGR_{\alpha M}$), there was still significant variation among grass host species in *Rhinanthus* performance after differences in host growth rate had been taken into account (likelihood ratio test: χ^2 = 6.2, P < 0.001, R^2 = 0.45), i.e. host grass species remained significant when fitted after the host grass growth rate. In particular, *Rhinanthus* had a substantially lower performance than expected when grown with *Anthoxanthum odoratum* and *Holcus lanatus* and a substantially higher performance than expected when grown with *Dactylis glomerata* (Fig. 4a).

Discussion

Our simple model assumes that the host plant actively takes up limiting nutrients and the parasite steals some fraction of this uptake; but, in doing so, it reduces host growth rate and hence further nutrient uptake. Thus, as is commonly observed, the combined mass of the host-parasite system is always lower than the mass of the host growing alone. We believe that this is the main reason for the observed reduction in yield in plant communities infected with parasitic plants, although the previous explanations for this effect − reductions in host photosynthesis (Cameron *et al.* 2008), inefficient nutrient use by parasites compared with hosts (Matthies 1995) and a shift in species composition towards species with lower growth rates (Bardgett *et al.* 2006) − can undoubtedly also contribute. Comparison of the relative contributions of these alternative mechanisms is a goal for future research.

As in population models with harvesting (e.g. fisheries), an intermediate level of harvesting maximises parasite yield (Nicholson 1954; Beverton & Holt 1957). Thus, an 'ideal' parasite would exercise prudent, rather than maximum, resource extraction. Variation in virulence among populations of *Rhinanthus* has been documented (Mutikainen *et al.* 2000) although a comprehensive study is lacking. The exact optimum virulence depends strongly on the host growth characteristics, but might also depend on the degree to which different individuals of the parasitic plants compete with each other, e.g. when multiple *Rhinanthus* individuals are attached to a single host plant, as is commonly observed (Prati *et al.* 1997; Westbury 2004). Competition among parasites selects for higher virulence because a prudent parasite no longer benefits from the under-utilisation of the host (Frank 1996).

Therefore, according to our model, the result of competition between parasitic plants would be 1) a reduction in the final, combined mass of the multiple parasitic plants, compared with the final mass of a single parasite, 2) an even greater reduction in the final biomass of the whole system (hosts plus parasites).

While it is often reported in the literature that *Rhinanthus* infects fast-growing grasses as preferred hosts (Ameloot *et al.* 2006; Bardgett *et al.* 2006), no previous study has tried to relate the performance of the parasite to the growth rate of the hosts. We found that *Rhinanthus* performance was strongly correlated with the growth rate of the host grass species, thus validating a key assumption of our model; however, *Rhinanthus* biomass on three of the nine grass species differed significantly from the predicted value suggesting that grasses may differ in their resistance to parasitism. While Cameron et al. (2006) have demonstrated substantial resistance in forbs, lignification of infected grass roots suggests that grasses may exhibit some degree of resistance (Rumer *et al.* 2007). However, it is also possible that the deviations in the performance of *Rhinanthus* on these three species may be due to differences in the growth rates of host grasses inside the glasshouse compared with outside in the garden.

Growth rates of host grass species.

Conventional measures of relative growth rate (RGR) are usually an average calculated over some common time interval. However, as the instantaneous growth rate expressed by an individual plant declines as it grows (Grime & Hunt 1975; Hunt 1982; Enquist *et al.* 1999), average growth rates measured in the usual way are heavily biased by initial size (Turnbull *et al.* 2008) and this bias could potentially mask important trade-offs, e.g., between growth rates in high vs. low light levels (Kitajima & Bolker 2003; Sack & Grubb 2003). We found that the rankings of species in terms of growth rate changed with light availability, although only to a limited degree, suggesting that such a trade-off is perhaps of limited importance. Surprisingly, there were more substantial cross-overs in species growth-rate ranks with increasing mass, although the relevance of this for coexistence is unclear.

The network model of West, Brown, and Enquist (West *et al.* 1997; 1999, WBE model) predicts that growth rate declines with plant mass to the ¾ power for most plants ($\alpha = 0.75$) with the exception of seedlings ($\alpha \approx 1$). When fitting these models to individual grass plants, we found that the best estimate of the common scaling exponent under 100% light was $\alpha = 0.879$ (95% CI = 0.871 – 0.888); and in the species-specific model α_i ranged from 0.825 (95% CI = 0.790– 0.860) to 0.904 (95% CI = 0.882–0.926). Grasses were grown from seed (and hence presumably could be classified as seedlings for some initial period) and this might explain why the measured values of α are higher than expected; however, the value of α also increases with increasing light. This is despite the fact that plants were much smaller in the low-light treatment and hence could presumably be classified as seedlings for longer. The growth of individual grass plants was also best described by a model in which α_i was species-specific, suggesting that the growth rates of different species (even those belonging to the same life-form) scale differently with mass.

Performance of Rhinanthus alectorolophus on different host grass species

The best predictor of *Rhinanthus* performance was absolute host growth rate calculated from a relatively simple model with a common scaling exponent α and by ignoring variation in the initial mass of the hosts. One possibility is that the initial growth rate model was overfitted, and this is a known danger when using the AIC for model selection (Anderson 2007). Overfitting implies

that some noise (non-information) has been included in the structural part of the model and the effects are not part of the actual process under study. Hence, including too many parameters makes the model so specific to the particular data set that prediction for new data sets is unreliable (Anderson 2007). That the best predictor of *Rhinanthus* performance came from a model ignoring variation in initial host mass could alternatively be due to a difference in the nature of host growth between the glasshouse and the garden. In the glasshouse, single plants were grown in large pots, and there was little evidence of resource restriction; however in the garden multiple grass seeds were sown. These seeds germinated quickly and grew during the autumn to form a dense sward and by early spring, plants already filled the pots. It is therefore possible that, due to resource restriction, growth was no longer limited by aboveground mass. In this case the exponent in eqn 1, α, might fall to 0, and the growth rate is given simply by β_i. For example, Turnbull *et al.* (2008) found that a model with a switch from exponential to linear adequately captured the growth of annual plants grown in small restricted pots.

Conclusions

We developed a simple model for the growth of a host plant coupled to a parasite (or hemiparasite) and tested a key assumption of this model. The model predicts that (1) the combined mass of the host-parasite system is always less than the mass of the host grown alone simply because, by removing host resources, the parasite reduces future resource uptake; (2) final parasite biomass should be greater when growing on host species with higher growth rates; and (3) *Rhinanthus* should adopt an intermediate virulence to maximise its own performance, although competition among parasites should select for increased virulence. All the grass species tested made good hosts for the parasite, and the major differences in parasite performance were explained by variation in host growth rates; however, there was evidence for some differences in resistance among host grass species. We conclude that extending and parameterising this model for different *Rhinanthus* populations could help to explain the variation in the effect of the parasite in different grasslands, for example grasslands differing in productivity.

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Chapter 4

Effects of Seed Predators of Different Body Size on Recruitment in Bornean Logged Forest

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Abstract

The Janzen-Connell hypothesis proposes that seed predation plays a major role in maintaining high levels of tree diversity in tropical forests. However, small and large predators differ in host specificity, size of home range and response to human disturbance. Logging and hunting could alter the relative importance of different seed predators by increasing the relative abundance of small versus large predators.

We manipulated seed density in plots beneath con- and heterospecific adult trees within a logged forest and excluded predators of different body sizes using cages. We show that small and large-bodied predators inflicted appreciable levels of seed predation but that they differed in their effect on con- and heterospecific seedling recruitment. In combination small and large-bodied predators dramatically decreased both con- and heterospecific seedling recruitment. In contrast, when larger-bodied predators were excluded small-bodied predators reduced conspecific seed survival favouring seedling recruitment of other species.

Our results suggest that seed predation influences initial recruitment, and that dipterocarp seed survival is affected differently by predators according to their body size. In the absence of larger predators, small predators may help to maintain diversity in degraded forest areas by selectively predating seeds of the maternal tree. Our work suggests that changes in the body size structure of the predator community in logged forests has the potential to change the patterns of seed predation that play a role in Janzen-Connell effects.

Keywords: Coexistence; density dependence; Janzen-Connell; maintenance of biodiversity; Sabah; seed predation; seedling establishment; tropical rain forest

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Abstrak

Hipotesis Janzen-Connell menyatakan bahawa pemangsaan biji benih memainkan peranan penting dalam mengekalkan tahap kepelbagaian pokok yang tinggi di hutan tropika. Walau bagaimanapun, pemangsa bertubuh kecil dan besar, berbeza berbanding daripada satu sama lain dalam pemilihan makanan, ruang tempat tinggal dan kepada reaksi activity manusia. Aktivity pembalakan hutan dan pemburuan boleh mengganggu proses ini dengan meningkatkan bilangan pemangsa bertubuh kecil berbanding pemangsa bertubuh besar.

Kami telah mengatur kepadatan biji benih dalam petak-petak penyelidikan di hutan terbalak dan mengecualikanpemangsa berlainan saiz tubuh dengan menggunakan sangkar. Kami mendapati bahawa, pemangsa bertubuh kecil dan besar menunjukan dengan jelas penukaran tahap pemangsaan kepada biji benih yang sama spesies dan perbagai species. Kami mendapati bahawa gabungan pemangsa bertubuh kecil dan besar, berdua-dua mengurangkan proses pertumbuhan biji benih yang sama spesies sama dan perbagai species. Walaubagaimanapun, kami juga mendapati bahawa apabila pemangsa bertubuh besar diasing, kami mengamati pemangsa bertumbuh kecil menyebabkan pokok yang kepunyaan species serupa turun dan meningkatkan process peningkatan biji.

Hasil kajian kami dapat menunjukkan bahawa kesan pemangsaan biji benih mengaruh process peningkatan biji,kami juga mendapati bahawa kehidupan biji benih dipterokap ter-

pengaruh oleh saiz permangsa. Jika pemangsa bertubuh besar tidak hadir, pemangsa bertubuh kecil mungkin membantu mengekalkan kepelbagaian pokok dalam kawasan hutan susut nilai dengan melakukan pemangsaan memilih biji benih spesies yang sama. Hasil kerja kami munkin menyatakan bahawa penukararan saiz komuniti pemangsa di hutan terbalak dapat menukarkan potensi pemangsaan biji dan mengaruh hipothesis Janzen-Cornnell.

Introduction

Human-induced changes to tropical ecosystems are manifold and a major threat to biodiversity. Currently, less than half of the original forests of South-East Asia remains and the levels of biodiversity are predicted to decrease by 42% during this century (Sodhi *et al.* 2004). In the state of Sabah, Malaysia, our study system, local wildlife populations are being depressed by hunting and are becoming depleted or extinct (Bennett *et al.* 2000). Understanding to what extent such changes to natural wildlife populations may affect forest dynamics with regard to seed-dispersal and seedling recruitment is an important requirement for the management of tropical forests. The Dipterocarpaceae represent 80-90% of the upper canopy of intact lowland forests in South-East Asia (Whitmore 1998; Turner 2001). Therefore, the impact of such changes on Dipterocarps may have particularly important consequences for the dynamics of Southeast Asian lowland forests.

Dipterocarps have evolved a reproductive strategy of interspecific synchronized seed production (mast-fruiting) once every 3 to 4 years interspersed by irregular periods of low seed production (Ashton *et al.* 1988). Mast-fruiting may be an evolutionary response that allows for the survival of seeds by satiating predators (Janzen 1974). Evidence suggests that mast-fruiting events may be driven by El Niño-induced droughts that trigger synchronous fruiting among dipterocarp tree species (Curran *et al.* 1999). However, logging, fragmentation, fire, over-exploitation and conversion to oil palm plantation have increased the frequency and intensity of El Niño-induced droughts, disorganising dipterocarp fruiting and inducing recruitment failure (Curran *et al.* 2004).

Several hypotheses have been suggested to account for the maintenance of high levels of tree diversity in tropical forests (Wright 2002). Of these, the Janzen-Connell hypothesis is the most widely accepted although its importance as a coexistence mechanism has not been clearly demonstrated (Hyatt *et al.* 2003; Gilbert 2005; Freckleton & Lewis 2006). The Janzen-Connell hypothesis suggests that species-specific predators could be a key factor in maintaining high levels of tree diversity in tropical forests. Janzen (1970) and Connell (1971) argue that species-specific predators concentrate their activities near adult trees where seed density is high (density effect) and cause higher mortality of seeds and seedlings near the maternal parent (species identity effect) than further away (distance effect). Reduced seed and seedling recruitment near conspecific adults inhibits the reproduction of abundant species, favours rare species recruitment and increases the probability of heterospecific establishment. This mechanism limits the potential of single species dominance in the community and could be a significant force in maintaining the high diversity of trees in tropical communities. Seeds and seedlings represent the most vulnerable stage in a tree's development (Harper 1977; Dalling *et al.* 1998; Comita *et al.* 2007) and density and distance-dependent processes are thought to occur most strongly during these early stages when individuals are most abundant and susceptible to higher

mortality (Howe & Smallwood 1982; Hammond & Brown 1998).

Logging and hunting in tropical rain forests cause changes in small and large predator densities and movement (Curran & Webb 2000; Wells *et al.* 2006) that may modify natural enemy effects on dipterocarp seedling recruitment. These pressures affect predators differently depending on body size. Small predators are less prone to hunting, they can persist in primary forest fragments and rapidly disperse into logged forest (DeMattia *et al.* 2004). Conversely, hunting pressure by loggers confines large predators to small primary forest fragments with no, or very low rates of, secondary forest recolonization (Peres 2001). Consequently, the decrease in large predator populations due to logging and hunting implies that small predator effect on tree recruitment may increase in relative importance. These changes have the potential to affect seedling recruitment in logged forest in fundamental ways and ultimately determine dipterocarp composition and diversity.

Although density and distance effects on seed and seedling survival have been widely studied in the tropics (reviewed in Clark & Clark 1984; Connell *et al.* 1984; Terborgh *et al.* 1993; Hammond & Brown 1998; Hyatt *et al.* 2003) and in dipterocarps (Blundell & Peart 2004; Stoll & Newbery 2005; Takeuchi & Nakashizuka 2007; Naito *et al.* 2008), no consensus has been reached over the general importance of the Janzen-Connell effect. A compilation of theoretical and experimental studies suggests that invertebrates support the predictions of the Janzen-Connell model, but vertebrates do not (Hammond & Brown 1998). We argue that vertebrate effects may change in relative importance with changes in the abundance of predators of different body sizes (DeMattia *et al.* 2004; Paine & Beck 2007). Indeed, because small and large predators have unique attributes (host preference, home range, behaviour, community structure, and population dynamics) effects predicted by the Janzen-Connell hypothesis may depend on the size of predators and on their potential contributions to seed mortality (Wells & Bagchi 2005). Dipterocarp predators in Bornean forests range from the large-bodied Bearded pig (57-83 kg) (Wong *et al.* 2005) to small rodents (<400 g) (Wells *et al.* 2006; Wells *et al.* 2008). Small-bodied predators include squirrels (*Ratufa affinis*, *Sundasciurus hippurus, Callosciurus adamsi, C. prevosti* and *C. notatus*) and rodents (*Maxomys surifer, M. rajah, M. whiteheadi, Leopoldamys sabanus* and *Sundamys muelleri*) (Natawira *et al.* 1986; Gould & Andau 1989; Curran & Leighton 2000; McConkey *et al.* 2002). They are usually specialists and cover a small home range that they rigorously inspect (Beck *et al.* 2004); they are therefore expected to specialise on seeds of particular species and to cause higher mortality near the maternal parents than further away independently of the density of seeds. In contrast large-bodied predators cover a wider home range, they are usually generalists and may travel long distances to feed on high density resources (Nakagawa *et al.* 2005; Wong *et al.* 2005). They are therefore expected to cause high mortality at high but not low seed density independent of species identity. Large-bodied consumers of dipterocarp seeds found in the Bornean forest include pigs (*Sus barbatus)* and macaques (*Macaca fascicularis* and *M. nemestrina*). Although pigs and macaques have been observed in the studied area, the population densities of these large species appeared to be low relative to primary forest perhaps due to hunting.

In this study we investigated the separate contributions of small and large predators to seed mortality in logged forest by comparing survival from uncaged control with cages that excluded large predators or both small and large predators. We focused on the seed-to-seedling transition. We show that, in the absence of large-bodied predators, small predators had a positive effect on

heterospecific dipterocarp seedling recruitment.

Methods

STUDY SITE AND SELECTION OF TREES. — This study was conducted during a partial fruiting event that occurred in February 2007 close to Taliwas, 25 km West of Lahad Datu, on the east coast of Sabah, Malaysian Borneo (4°58' N, 118°06' E). This lowland tropical secondary forest was logged in the early 1970s (Marsh & Greer 1992) and is dominated by trees from the family Dipterocarpaceae. Dipterocarps produce fleshy single-seeded fruits that are dispersed by wind, water or occasionally by animals. The seeds are recalcitrant, meaning that they germinate within days of dispersal and display no seed bank (Curran *et al.* 1999). The site was located in an alluvial plain and covers an area of 5.4 ha. This area experiences a wet equatorial climate. The temperature and precipitation are comparable to the Danum Valley Field Centre (Marsh & Greer 1992; Wong *et al.* 2005) where mean annual temperature is 26.7°C, mean maximum temperature is 30.9°C and mean minimum is 22.5°C. Average rainfall is about 2700 mm per year, although severe droughts regularly occur influenced by the El Niño Southern Oscillation (ENSO) events that were recorded during 1986–87, 1991–94 and 1997–98.

In the beginning of February 2007, we selected ten easily accessible fruiting adult trees (169-370 cm DBH) belonging to five species of Dipterocarpaceae, between the 13th and 17th km on the logging road from Lahad Datu to Taliwas: *Parashorea tomentella, Shorea leprosula, S. parvistipulata, S. johorensis, Dipterocarpus caudiferus*. Trees were situated between 138 m and 182 m above sea level and between 21.1 m and 139 m from the logging road (see Table 1 for a complete description of the trees). All trees were located in logged forest except one individual of *S. leprosula* that occurred in a *Nephelium lappaceum* L. (Rambutan) and *S. leprosula* plantation.

EXPERIMENTAL DESIGN. — Three types of exclosures were designed to exclude the access of small or large predators. The name of each exclosure indicates the size class of mammals that were permitted access. Small and large mammals were excluded (NONE) using wired mesh cages (1 x 1 m, 0.5 m tall and 1 cm mesh size). The effect of small predator effect was isolated using identical exclosure cages to NONE but with four openings along each side of the cage (10 x 10 cm) that excluded large predators allowing only small predators access (SMALL). Small and large predators were allowed to access an open control (ALL). Exclosures were secured at their base with spikes so that the mesh was tight against the ground. This experiment was an incomplete factorial design because it was not feasible to exclude small predators and not exclude large predators at the same time.

Freshly fallen seeds were collected from under the canopy of the selected trees one to two days before the experimental set up. Seeds with indications of predation, fungal damage or germination were discarded. The biomass of the seeds used in the experiment was predicted using regressions relationships established for each species from 20-50 additional seeds dried at 80°C to constant mass. The average seed weight of species used in this experiment ranged from 0.7 - 6.5 g (Table 1).

Table 1. Characteristics of the study trees. DBH: diameter at breast height.

Tree pair	Tree species	DBH (cm)	Seed estimation on the 7.02.07	Seeds collected between the 10th and the 20th of February 2007	Mean seed biomass (g)	Distance between the pairs (m)
1	*Parashorea tomentella* (Sym.)	240	800-1000	284	4.0	589
1	*Shorea leprosula* (Miq.)	200	3000-5000	903	0.6	
2	*Shorea parvistipulata* (Heim.)	216	300-400	118	1.8	558
2	*Shorea johorensis* (Foxw.)	370	3000-5000	855	0.9	
3	*Shorea johorensis*	250	3000-5000	2286	1.0	878
3	*Shorea leprosula*	169	3000-5000	858	0.7	
4	*Dipterocarpus caudiferus* (Merr.)	225	300-400	126	6.5	798
4	*Shorea leprosula*	174	3000-5000	135	0.7	
5	*Shorea parvistipulata*	307	3000-5000	461	1.3	521
5	*Shorea leprosula*	184	3000-5000	775	0.6	

The Janzen-Connell effect predicts high seed mortality by specialist seed predators close to the maternal tree where seed density is high. Therefore, to estimate both the con/hetero-specific and density effects we swapped seeds from pairs of trees belonging to different species into high and low density treatments. The con/hetero-specific effect inevitably includes a distance effect. Trees were paired in a way that minimised the difference in the distance between the five pairs of trees (between 521 and 878 m). To estimate the con/hetero-specific effect, for each tree in the pair, seeds coming from the conspecific tree (seeds belonging to the maternal tree of the pair) and from the heterospecific tree (seeds belonging to the distant paired tree of a different species) were placed within each exclosure at the same density of seeds (crossed experimental design, see Fig. 1). To estimate the density effect, for each exclosure type seeds were placed at either high density (corresponding to natural density: 24 seeds/m^2, 12 con- and 12 hetero- specific) or low density (2 seeds/m^2, 1 con- and 1 heterospecific)

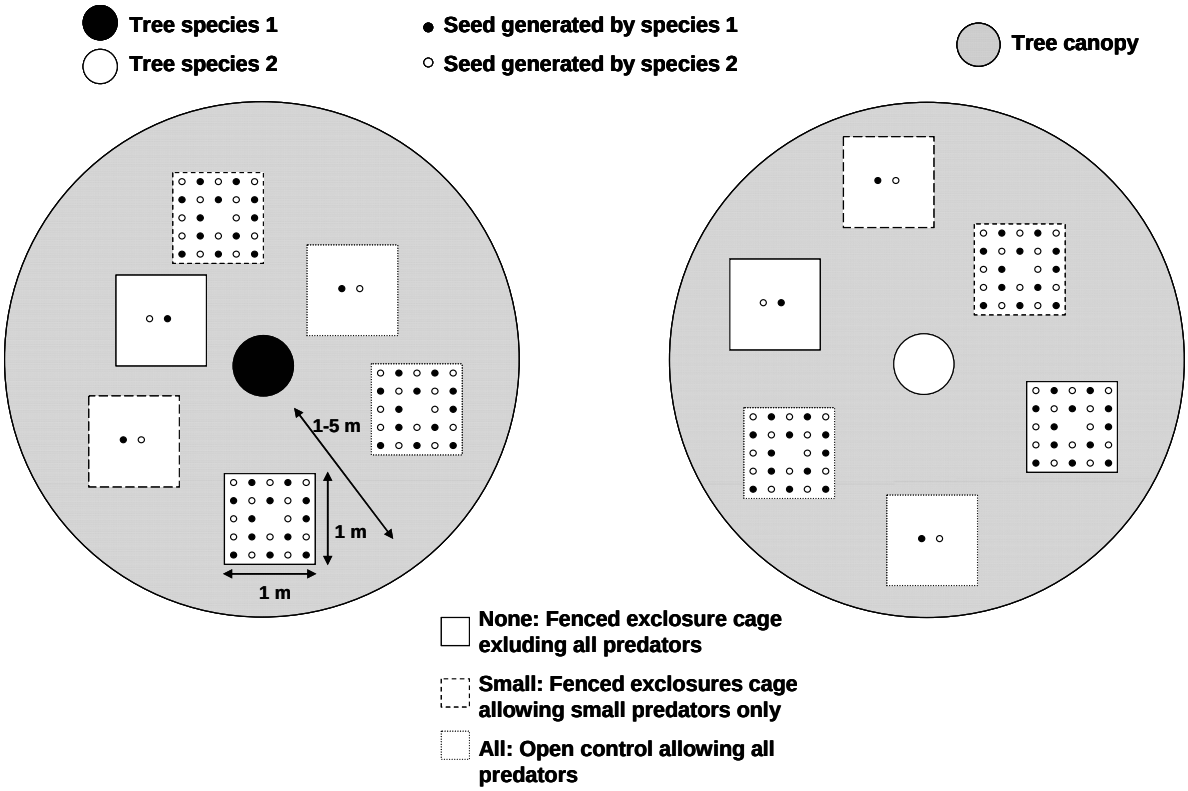

Figure 1. Seeds of each pair of trees (5 pairs) were placed between 1 and 5 m around each tree at high density (24 seeds, 12 seeds from the maternal tree (conspecific) and 12 seeds from a distant tree of a different species (heterospecific) and at low density (2 seeds, 1 con- and 1 hetero-specific). The experimental design consists in three exclosure treatments (1 x 1 m large x 0.5 m tall): (1) None, fenced exclosure cage excluding both large and small predators, (2) Small, fenced exclosure cage excluding only large predators and (3) All, open control allowing both small and large predators.

(Maycock *et al.* 2005). Seeds were placed 15 cm apart (see Fig. 1). We followed the fate of the seeds by tethering each seed with a 3 m string to a nail dug into the soil at the position of the seed. Exclosures were placed randomly within a radius of 1-5 m randomly from the trees.

We estimated the number of seeds on the ten fruiting trees visually with binoculars at the time of seed collection (Table 1). Background seed densities under the canopy of the parent tree were determined from seeds that fell on the experimental cages (four cages of 1 x 1 m per tree) and in three litter traps of 1 x 1 m placed at breast height and set up randomly at 2, 3 and 4 m from each tree. Moreover, to prevent large differences in the background seed densities between the selected trees interacting with the density treatment, every three days during the period of the experiment freshly fallen seeds were collected on the soil below the tree canopy where the density was highest.

The majority of dipterocarp seeds germinate within days of dispersal (Itoh 1995). Seeds were monitored on day 18 and scored as alive, germinated or missing. We used removal as an indicator for seed predation assuming that seeds found missing were either immediately eaten at tethered locations or some time later if they were cached by rodents (Jansen *et al.* 2002). Missing seeds, gravely damaged seeds and seeds that did not germinate were therefore scored as dead.

On the other hand, germinated seeds that were intact or slightly damaged were scored as alive.

STATISTICAL ANALYSIS. — We used generalized linear mixed-effects models (GLMMs) (Bates 2005; Faraway 2005; Gelman & Hill 2007; Maindonald & Braun 2007; Bolker *et al.* 2009), with a binomial error distribution since our design includes fixed and random effects and seed mortality is a binary response variable. Generalized linear mixed-effects models (GLMMs) are generalized linear models (GLMs) that include random effects. The GLMMs were fitted using maximum likelihood (ML) and restricted maximum likelihood (REML) with the lmer function from the lme4 library (Bates 2005) for R 2.8.1 (R Development Core Team 2008). For the analysis of seed survival, we assessed the importance of interaction terms using Akaike's information criterion (AIC). The predator size effect ("exclosure"), the con/heterospecific effect ("seed identity") and seed density effect ("density") were treated as fixed effects. We also assessed the importance of the interaction term between predator access and covariates for the background seed density on seed predation. Tree species and plots were treated as random effects. In the text and figure, we present point estimates of the means and slopes from the GLMMs with their standard errors.

Results

After 18 days, body size of seed predator, seed identity (con- or heterospecific seeds) and seed density had an interactive effect on seed survival (Fig. 2A & 2B; Table 2). In exclosures from which all the predators were excluded seed survival was relatively high at both high and low densities (on average between 80 and 100%) and was comparable between con- and heterospecific seeds (Fig. 2, bottom row). In contrast, in exclosures open to predators their effects on seed survival differed with seed densities and identity. Small predators reduced conspecific survival at high but not low seed density whereas small and large predators combined reduced conspecific survival at both high and low seed density and heterospecific survival at high but not low seed density.

At high seed density (Fig. 2A, left hand side), small predators did not affect heterospecific survival (seeds from the tree of the other species) whereas conspecific survival (seeds from the maternal tree) was reduced by about half, from 80 percent (65 - 90) to 39 percent (22 - 59). This difference between con- and heterospecific seed survival was cancelled out in exclosures in which small and large predators were allowed since they inflicted relatively high seed mortality independent of the identity of the tree (29 percent (16 - 47) heterospecific survival and 35 percent (26 - 54) conspecific survival).

At low density (Fig. 2B, right hand side), seed survival was relatively high in both exclosure types. Seed survival was not affected by small predators alone. In contrast, when all seed predators were allowed access (small and large) there was lower survival of conspecific seeds compared to heterospecific seeds, reducing conspecific recruitment by 60 percent, from 74 percent (56 - 86) to 26 percent (14 - 44).

Discussion

Logging and hunting pressures in tropical rain forests are leading to reduced populations of large seed predators. As the mechanisms maintaining dipterocarp diversity in logged forest areas may depend on the size of seed predators (Maycock *et al.* 2005; Paine & Beck 2007), the decrease in large seed predator populations may increase the importance of small seed predators on dipterocarp recruitment. Here we show that small seed predators specifically selected seeds from the maternal tree and therefore increased recruitment of seeds from other

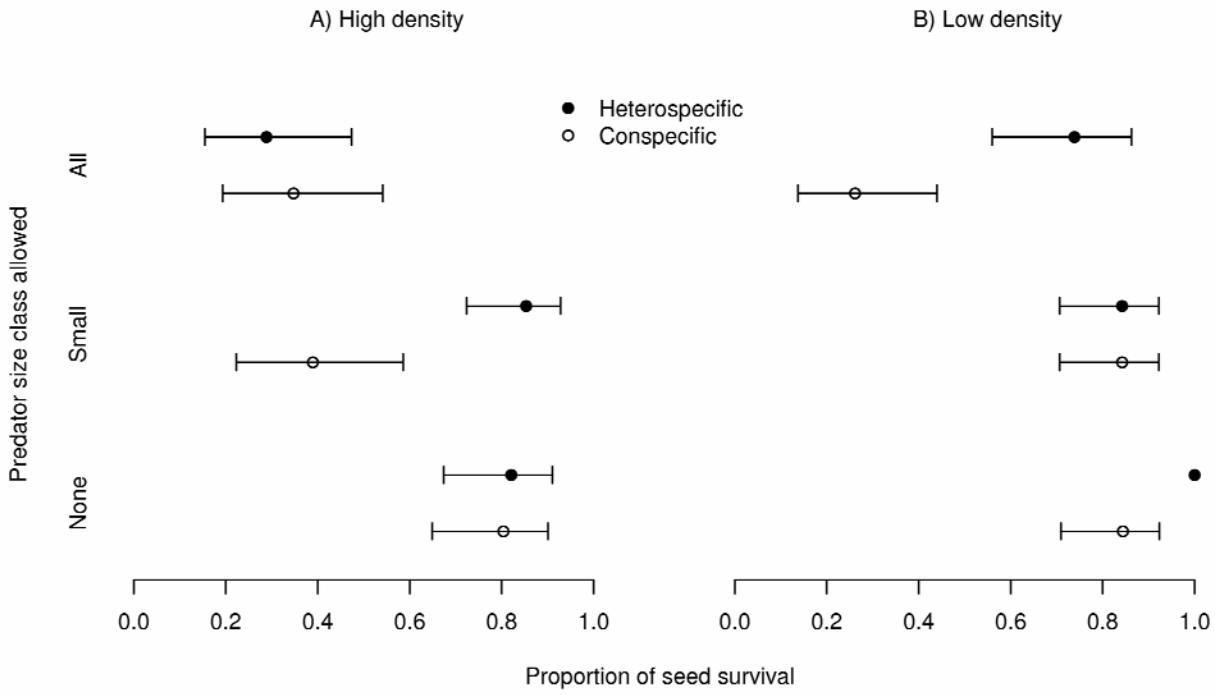

Figure. 2. Percentage of mean seed survival in exclosures that allowed predators of the specified size classes close (conspecific) or away (heterospecific) from maternal tree for (A) the high density treatment and (B) the low density treatment. Results are shown as means ± s.e.m. back transformed from the generalized linear mixed-effects model analysis. Note that the estimates for heterospecific seeds in the treatment excluding all predators (None) at low density are effectively zero at the resolution of the figure.

Table 2. Evaluation of mixed effect models of A) the three-way interaction term of exclosure cages, seed specificity and seed density B) the two-way interaction term of exclosure cages and background seed density, on seed survival tested by fitting a model with and without this interaction term. Table shows the output from the GLMM statistical analysis giving log-likelihood, information criteria values (AIC), model probabilities (ωi) and evidence ratios (E).

Model	Df	AIC	logLik	ωi	*E*
A. y ~ exclosure cage * seed specificity * seed density					
without the three-way interaction	12	857.7	-416.9	0.918	1.000
with the three-way interaction	14	852.9	-412.4	0.082	11.190
B. y ~ exclosure cages * background seed density					
without the two-way interaction	7	932.6	-459.3	0.243	3.774
with the two-way interaction	9	934.8	-458.4	0.757	1.000

species, an effect that is eliminated when large seed predators have access to the seeds.

Small seed predators decreased recruitment of seeds from the mother tree (conspecific) at high but not low density. Small seed predators are usually specialists, ubiquitous and occupy small, sometimes non-overlapping territories (Beck *et al.* 2004). They may prefer local seeds and be able to discriminate between seed species based on their size (Bodmer 1991; Blate *et al.* 1998; Jansen *et al.* 2002; DeMattia *et al.* 2004). This behaviour may explain the result observed at high seed density: when small seed predators were allowed access they consumed disproportionately more seeds from the maternal tree, leaving seeds coming from the distant tree of a different species. On the other hand, because in their small territory small seed predators rigorously inspect the forest understory we might expect to observe the same pattern at low density, but we do not. The low predation rate at low seed density could be explained by satiation of the small seed predators. Indeed, in addition to the number of seeds experimentally placed around the trees, the number of background seeds collected under the tree canopies during the experiment ranged between 118 and 2286. Small seed predators might have initially concentrated on the high seed density treatment and on the background seeds. These may have satiated predators before they could exploit the less profitable low density plots.

In contrast to small seed predators, large seed predators dramatically reduced seed recruitment of conspecific seeds at low density. Unlike small seed predators, larger species are generalists and occupy large home ranges where they may travel long distances to feed on high density resources (Nakagawa *et al.* 2005; Wong *et al.* 2005). This behaviour could explain why seed survival was greatly reduced at high density independently of the seed species identity. However, at low density we expected that large seed predators would neglect seeds and therefore allow high survival of both con- and heterospecific seeds. Because both small and large seed predators had access to the open control, this higher predation on conspecific seeds at low density could be due to small seed predators. Indeed, small seed predators might choose to enter the exclosure cages only in case of a high payback. This would explain the results observed at low seed density, with the small seed predators not entering into the exclosure, but instead predating conspecific seeds in the open control.

The background seed density may interfere with the experimental density treatment to affect seed survival; a higher background seed density may attract more predators and therefore inflict lower survival independently of the density treatment. In our study seed predator size classes did not interact significantly with the background seed density (non-significant two-way interaction term, Table 2). This suggests that our density treatment was not influenced by the background seed density.

Our results show that small seed predators caused disproportionately higher mortality of seeds from the maternal tree, generating an advantage for establishment of seeds from other dipterocarp species. Furthermore, they appear to prefer areas of high density, suggesting that they might be optimising their foraging on a local scale. This process could, as suggested by the Janzen-Connell hypothesis, promote diversity at both the landscape and local scales. Predation by large mammals on the other hand, appears to be independent of density, at least at the scale examined. This may reflect that large seed predators respond to density at a landscape scale, as has been previously suggested (Curran & Leighton 2000). Once they discover a seed shadow, they proceed to exploit it very thoroughly. Why large predators appear to discriminate between species at low but not high density is unclear.

Interestingly, at one of the sites we observed that a fiddler crab had carried seeds into its hole and this species may be a previously undiscovered disperser and predator of dipterocarp seeds (Fig. 3). Crabs may therefore be important for local dispersal of freshly fallen seeds as has been shown recently in the neotropics (Capistran-Barradas *et al.* 2006).

A meta-analysis based on 40 papers and 75 species reported that even though distance-dependent survival is evident for some species, the data available did not support the Janzen-Connell hypothesis to be a general phenomenon across communities (Hyatt *et al.* 2003). By separating the individual effects of small and large seed predators our results may explain why outcomes of previous experiments testing the Janzen-Connell hypothesis have been so disparate. Varying results from studies of the Janzen-Connell effect might arise in part from the structure of the seed predator community and whether it has been affected by human activities (logging, hunting, etc) (Wright *et al.* 2000; Wright & Duber 2001; Wyatt & Silman 2004). Due to their specialisation and small territory occupation small seed predators can create an advantage for heterospecific seedling recruitment success which is the key of the Janzen-Connell hypothesis. However, if the effect of small seed predators is confounded with that of large seed predators, usually the case in natural ecosystems, the evidence for distance-dependence may disappear. Therefore, our results reveal the relative importance of small seed predators in primary forest, logged forest or forest fragments when larger seed predators are lacking.

Our short term study focussed on the seed to seedling transition and does not investigate the entire process of dipterocarp recruitment. However, several lines of evidence suggest that the positive effect of small seed predators on heterospecific dipterocarp recruitment in logged forest might be prolonged in the longer term. First, several studies have shown that seed predation is higher in logged forest than in primary forests and therefore a strong driver of recruitment process in logged forest (Hammond 1995; Hammond & Brown 1995; Howlett & Davidson 1996; Notman *et al.* 1996; Curran & Webb 2000; Wright *et al.* 2000; Wright & Duber 2001; Peña-Claros & De Boo 2002). Second, although high seed predation and poor germination limit establishment in logged forest, it has been shown that seedling grow well in logged forest compared to primary forest (Howlett & Davidson 1996). Third, pigs and rats have been shown to avoid germinated seeds (Curran & Webb 2000). Our experiment manipulated seed density and species identity at a relatively small scale that might have favoured small versus large seed predator effect, however our results are consistent with those recently found in neo-tropical forests (Paine & Beck 2007).

Large scale masting events in Borneo appear to become less frequent, with changes to precipitation patterns, and hence increasing drought frequency, the most likely explanation (Curran *et al.* 1999; Curran & Webb 2000; Sakai *et al.* 2006). Failure of major mast fruiting events are accentuating food scarcity leading to fragmented forests lacking large seed predators (DeMattia *et al.* 2004; Wong *et al.* 2005). According to our results, small seed predator choice of specific seeds is crucial when populations of large seed predators are missing. The findings of this study may thus be of particular importance for forests that are lacking large seed predators.

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Figure 3. Fiddler crab (arrowhead) halfway in its hole with the strings and seeds going into the hole. Photo credit: Yann Hautier.

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General discussion

In this thesis, I experimentally test some of the mechanisms responsible for the maintenance or loss of plant diversity. In Chapter 1 and 2, I manipulated the productivity and light availability in the understory of grassland communities to investigate the importance of asymmetric competition for light and its consequences for plant competitive dominance and exclusion. Moreover, I explored the potential use of the manipulation of the importance of competition for light via frequent cutting and sucrose addition as a tool for grassland restoration (Chapter 1). I also investigated the role of negative feedbacks by natural enemies on the maintenance of diversity in grasslands and tropical forests (Chapter 3 and 4). In particular, I examined the relationship between hemiparasitic plant performance and host growth rate (Chapter 3). Finally, I tested the importance of seed-predators of different body size in the recruitment process of dipterocarp seedlings within a tropical logged forest (Chapter 4). Detailed discussions of results are given at the end of each chapter.

The importance of competition for light for species coexistence

I generally found strong evidence that asymmetric competition for light is an important driver of competitive exclusion and diversity loss under productive conditions. My results show that understory light availability in our unproductive communities was relatively high over the growing season and that the importance of competition for light was reduced compared with productive communities (Chapter 1). Under unproductive conditions competition was mainly belowground for limiting nutrients. Plants competed proportionally to their size for the contested soil resource, a so called size-symmetric process that did not lead to diversity loss (Chapter 2). I demonstrated that eutrophication generally increased biomass production, decreased understory light availability over the growing season and increased the importance of aboveground competition for light (Chapter 1). Under productive conditions competition was mainly aboveground for light. Plants competed disproportionately to their size for the contested light resource, a size-asymmetric process that led to diversity loss (Chapter 2). Together, my results confirm the idea that when ecosystems receive sufficient light plants compete primarily for limiting nutrients, while with ample nutrients plants compete primarily for limiting light (Weiner 1990; Casper & Cahill 1996; Casper & Jackson 1997; Blair 2001). Moreover, they show that while asymmetric competition for light did not lead to disproportionate competitive exclusion and loss of diversity under unproductive conditions, nutrient inputs exacerbated the importance of asymmetric competition for light that drove the decline of diversity.

Is this increased strength of competition for light the mechanism of diversity loss with eutrophication? The evidence of the underlying mechanism came from the following methodology. By applying additional understory light together with fertilization I was able to mitigate the increased shading caused by the greater aboveground biomass

production and to prevent the loss of species diversity. Moreover, by comparing transplanted seedlings planted in plastic tubes to reduce belowground competition to seedlings exposed to full root competition, I was able to show that belowground competition did not play a role in this loss of biodiversity (Chapter 2). My experimental manipulation of light is the first direct demonstration of the importance of asymmetric competition for light as a mechanism of plant diversity loss, while earlier work has been largely correlational, or experimental but indirect and with potential side-effects (Emery *et al.* 2001).

Restoring grassland diversity

Although the results of my first two chapters suggest that there is no easy remedy to counteract the negative impact of fertilization, they suggest that the importance of competition for light can be manipulated and used as a tool for restoring plant diversity to eutrophied grassland ecosystems. For example, carbon amendments to eutrophied soil (Blumenthal 2003; Baer *et al.* 2004; Corbin & D'Antonio 2004), clipping regime (Lulow 2008; Tang *et al.* 2009) and naturally occurring hemiparasites (Davies *et al.* 1997; Pywell *et al.* 2004; Bullock & Pywell 2005; Westbury *et al.* 2006) have been used as restoration tools to increase species diversity. I have shown that carbon addition increased understory light availability through reduced nutrient availability and increased belowground competition (Chapter 1). I have also shown that frequent cutting increases light availability to subordinate plants (Chapter 1) and it has been demonstrated that repeatedly removing the hay decreases nutrient availability in the long term (Felske *et al.* 2000). My results suggest that frequent cutting and carbon addition to soil might restore plant diversity by increasing light availability to subordinate plants. This mechanism might in turn reduce the importance of competition for light by preventing initial dominance pattern from being maintained and avoiding light intercepting ability dictating the outcome of competition over the growing season. Although an increase in understory light availability through the reduction of fast-growing grass productivity by hemiparasites (Chapter 3) could be a mechanism of maintenance of diversity, to my knowledge experiments measuring light availability in relation to the presence of hemiparasites are missing.

On the other hand, maximizing the importance of competition for light in agricultural landscape could be used to competitively suppress undesirable weeds. For example, increased crop density and uniformly distributed crop in two-dimensional space rather than sown in traditional rows (Weiner *et al.* 2001) provide an alternative to mechanical weed control in organic farming and reduce environmental impacts of agriculture by reducing herbicide application in conventional farming (Weiner pers. comm.).

Negative feedbacks and the maintenance of diversity

I also explored the mechanisms by which negative feedbacks by natural enemies act as a regulator of diversity in grasslands and tropical forests. A meta-analysis showed that the total combined biomass of hosts and parasites is reduced by ~25% compared with controls (Ameloot *et al.* 2005). This reduction is usually attributed to lower nitrogen-use inefficiency by the parasite compared with the host (Matthies 1995; Ameloot *et al.* 2005) or to reductions in host photosynthesis (Watling & Press 2001; Cameron *et al.* 2008). My model of host-parasite interaction (Chapter 3) proposes an alternative explanation to explain how the combined mass of the host-parasite system is always reduced compare to the host grown alone. This is because host plants, and not the parasite, are able to directly uptake resources. As these resources limit system productivity, the system biomass is dependent on host growth

rate only. Therefore, the extraction of resources from the host by the parasite leads to reduced future resource extraction by the host and hence reduced system productivity. Hemiparasites such as *Rhinanthus alectorolophus* germinates in the early spring and is present for the active growth phase only, when host biomass is strongly increasing. In my model, the key assumption is that parasite growth rate and hence parasite performance is a function of host growth rate. I validated this assumption by showing that *Rhinanthus* performance was strongly correlated with the growth rate of the host grass species. Reducing fast-growing grass productivity is likely to lead to a decrease in their competitive dominance that may benefit to subdominant forbs. The competitive release of parasitized grass species is therefore likely to be a mechanism of maintenance of grassland diversity.

Further, I have shown that in Bornean tropical forest small predators selected seeds belonging to the mother tree and therefore increased recruitment of seeds belonging to distinct trees, an effect that is hidden when large predators have access to the seeds (Chapter 4). This finding shows that small predators may help maintain diversity in primary forest, logged forest or forest fragments that are lacking large predators. Together, these results demonstrate the importance of natural enemies in the maintenance of plant diversity.

Suggestions for future research

While the results of the short-term experiment I report in chapter 1 are consistent with recent longer-term findings, they did not provide a clear cut result on the importance of belowground competition. This is because my study could not test for limitation by all potential resources and so an additional role of other forms of competition cannot be completely discarded. Moreover, although all pairwise combinations were tested, my findings are based on five grass species. Additional experiments should clearly analyze the relative importance of both above and belowground competition on the outcomes of competition in mixed grassland communities that include the three common functional groups grass, forbs and legumes.

The methodology applied in chapter 2 provides a significant advance in our understanding of the mechanism of competition along nutrient gradients. It not only provided new insights on the relative importance of above and belowground competition, but may also inspire a new generation of mechanistic research that tests how widespread this result is in nature and how strong the effect is compared to other causes of diversity loss (e.g. acidification, litter accumulation).

I have shown that the size-corrected growth rate analysis of chapter 3 is a powerful method that produces meaningful parameters representing informative characteristics of the intrinsic growth potential. My application of this new method revealed a potential mechanism of grassland restoration by the generalist hemiparasite *Rhinanthus alectoroplophus*. My study focussed on grass species that are reported to be preferentially infected. However, experiments that test the effect of parasites on the competition between plant species of different functional groups are required to clearly understand the mechanism by which they affect the structure and diversity of grassland communities.

Finally I show that the changes in small and large predator densities might impact the recruitment of dipterocarp seedlings (Chapter 4). Although, my short-term study was conducted on a local scale with a small numbers of tree species, it provided promising results that should stimulate new long term research that separate the effect of small and large predators and manipulate wide range of tree species on a broad scale.

Conclusion

This thesis clearly demonstrated that fertilization leads to a shift from belowground

competition for limiting nutrients (a symmetric process that allow species coexistence), to aboveground competition for limiting light (an asymmetric process) leading to disproportionate species dominance and diversity loss. The consequence for grassland management is that there is no easy 'cure' to counteract the negative effects of eutrophication. Rather we should avoid nutrient enrichment to preserve biodiversity. However, I have shown that manipulation of the importance of asymmetric competition for light through carbon addition, frequent cutting and parasitism is a potential tool for restoration of eutrophic grasslands.

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Books

Randin, CF, Pearman, PB, Broennimann O, **Hautier Y** and Lecomte N. La compétition interspécifique. In: Thomas F, Lefevre T and Raymond M (Eds) Biologie Evolutive (Evolution des interactions entre espèces). De Boeck.

Poster presentations

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Oral presentations

2009 **Hautier Y**, Niklaus PA and Hector A. Competition for light causes plant biodiversity loss after eutrophication. 94th ESA Annual Meeting, Albuquerque, USA.

2009 **Hautier Y**, Niklaus PA and Hector A. Competition for light causes plant biodiversity loss after eutrophication. 2nd European Congress of Conservation Biology, Prague, Czech Republic.

2009 **Hautier Y** and Hector A. Mechanisms of maintenance and restoration of plant diversity. University Cork College, Cork, Ireland.

Professional recognition and awards

2007 Third price of the Poster Award of the Symposium of the Zurich-Basel Plant Science Center. Zurich, Switzerland. Effects of predator body size on the mortality of tropical forest tree seeds.

2008 First price of the Poster Award of the Biology08 meeting. Lausanne, Switzerland. Effects of predator body size on the mortality of tropical forest tree seeds.

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International collaborations

As participant of the Nutrient Network (NutNet) Global Research Cooperative, I coordinate the Swiss site in Früebüel, Zug, Switzerland and together with Eric Seabloom and Andy Hector leader of the project “Diversity-productivity relationships in grasslands: a global comparison”.

Manuscript reviews for

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Teaching (since 2009)

Master	Introduction to R
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Selected media coverage

Radio	L’actualité des sciences; Swiss Radio RSR.
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Annex

Diversity and stability: a multisite test of the insurance hypothesis using experimental grassland communities

Andy Hector, Yann Hautier, Philippe Saner, Luca Wacker, Robert Bagchi, Jasmin Joshi, Michael Scherer-Lorenzen, Eva M. Spehn, Ellen Bazeley-White, Maja Weilenmann, Maria C. Caldeira, Panayiotis G. Dimitrakopoulos, John A. Finn, Kerstin Huss-Danell, Ari Jumpponen, Christa P. H. Mulder, Cecilia Palmborg, João S. Pereira, Akis S. D. Siamantziouras, Andrew C. Terry, Andreas Y. Troumbis, Bernhard Schmid and Michel Loreau (*Ecology* in Revision)

Abstract

An insurance effect of biodiversity occurs when the functioning of more diverse ecosystems is buffered against temporal and spatial variability by the asynchronous fluctuations of the populations of individual species. For example, when the responses of different species are not perfectly positively correlated declines in some species are compensated by increases in others which smoothes the collective productivity of the whole community. This insurance effect of biodiversity has been likened to the risk-spreading benefits of diverse investment portfolios in financial markets.

We use data from a co-ordinated network of grassland biodiversity experiments to perform a general test of the insurance hypothesis by examining the effect of plant diversity on the temporal and spatial variability of individual species, functional groups and communities at multiple sites. We tested the hypothesis that diversity has a stabilizing effect on the biomass production of the aggregate community and investigated the mechanisms that are thought to generate this insurance effect.

Our results support a stabilizing effect of diversity on temporal variability. This stabilizing effect of diversity came about largely through asynchronous fluctuations of the populations of individual species, which were less stable in more diverse communities. Overyielding also contributed to stability by increasing mean biomass production relative to its variability. In combination with previous work, our results suggest that stabilizing effects of diversity on community productivity appear to be a general phenomenon in grassland ecosystems.

Keywords: Biodiversity; Productivity; Ecosystem functioning; BIODEPTH project; Portfolio effect; Overyielding

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Introduction

One possible value of biodiversity to humans is its potential to buffer ecosystem processes like productivity against environmental variation. This insurance value (Yachi and Loreau 1999) of biodiversity has usually been considered in the context of fluctuations over time, where it has been likened to the risk-spreading benefits of diverse portfolios of investments in financial markets (Doak et al. 1998; Lehman and Tilman 2000; Tilman 1999; Tilman et al. 1998), but could also apply to spatial environmental variation (Loreau et al. 2003). This insurance effect requires only that fluctuations in the abundances of a guild of species are not perfectly synchronised, because under perfect synchrony an entire guild or trophic level would effectively behave as one species. When species responses are not perfectly positively correlated changes in some species can be compensated by others and the averaging of their asynchronous fluctuations smoothes the collective productivity of the whole community (Doak et al. 1998; Tilman et al. 1998; Yachi and Loreau 1999). Stabilizing effects of biodiversity can also result through overyielding if higher levels of diversity increase mean levels of ecosystem functioning (e.g. biomass production) relative to the variation in functioning (see methods).

One potentially confusing or counter intuitive aspect of the insurance hypothesis and of the portfolio effect is that diversity can have a stabilizing effect on aggregate community or ecosystem properties (like primary productivity) at the same time as the

fluctuations of the constituent species may be destabilized due to interactions with greater numbers of species (Tilman 1996). This destabilization of species populations by diversity is not an essential feature of the insurance hypothesis but may partly explain why both stabilizing (on ecosystem processes) and destabilizing effects (on populations) of diversity have been reported during the decades of research on the relationship between diversity and stability. With respect to the insurance hypothesis, the key point is that a stabilizing effect will occur whenever the fluctuations of individual species are not perfectly positively correlated. This asynchrony through differential species responses can be interpreted as a form of temporal niche differentiation (Loreau 2000).

Stabilizing effects of diversity can also arise when diversity increases levels of mean ecosystem functioning relative to the variability in ecosystem functioning. For example, complementarity effects (Loreau and Hector 2001) lead to overyielding of plant biomass production: levels of productivity that are greater than expected based on the performance of species in monoculture. A recent meta-analysis of 44 biodiversity experiments (Cardinale et al. 2007) found that complementarity effects were widespread and accounted for nearly two thirds of the effects of diversity on production (with selection effects accounting for the remaining third). If these positive effects of diversity on levels of mean productivity outweigh any changes in the variability of production then overyielding can also contribute to the insurance effect.

Ives & Carpenter (2007) recently reviewed the diversity-stability literature emphasizing its breadth and complexity due to, amongst other things, the many different types of stability and the range of different variables that stability measures can be calculated for (e.g. stability of population abundance *vs* total community biomass as introduced above). For experiments where diversity was directly manipulated they report two positive effects of plant species diversity on the stability of biomass production (Caldeira et al. 2005; Tilman et al. 2006); to which we would add (Pfisterer et al. 2004; Seabloom 2007; van Ruijven and Berendse 2007) and three positive effects of microbial diversity on the stability of biomass (Steiner et al. 2005; Zhang and Zhang 2006) or carbon dioxide production (Morin and McGrady-Steed 2004). Their review contains no reports of negative or neutral effects of diversity on temporal stability of ecosystem processes from grassland experiments, but one negative effect of increased multitrophic diversity on the temporal stability of biomass production in seagrass beds (France and Duffy 2006), and one neutral (Petchey et al. 1999) and one negative effect (Gonzalez and Descamps-Julien 2004) of microbial diversity on the stability of microbial biomass production. In the field, observational studies have also looked at stabilising effects of biodiversity on ecosystem processes with Ives & Carpenter reporting five positive effects of plant diversity on temporal stability (Bai et al. 2004; Dodd et al. 1994; Leps 2004; Seabloom 2007; Tilman 1996; Valone and Hoffman 2003) and one neutral effect (Leps 2004). On the other hand, recently (Polley et al. 2007) found no stabilizing effect of diversity in remnant and restored prairie communities because temporal variability of aboveground biomass production was driven by that of the dominant species.

In this paper, we provide the first general experimental test of spatial and temporal insurance effects of diversity within a single trophic level (grassland plant communities) using the BIODEPTH network of coordinated biodiversity experiments conducted at eight European grassland field sites. We test the effects of biodiversity on the stability of species, functional groups and whole communities in both time (over three years) and space (between the paired replicates of the experimental plant communities

grown at each site).

Materials and Methods

Data

The analyses presented use data on net aboveground biomass production (g m^{-2} $year^{-1}$) of species from the experimental plots at each of the eight BIODEPTH fieldsites for the three main years of the project (Hector and Bagchi 2007; Hector et al. 1999; Spehn et al. 2005). In total the dataset contains information on 480 plots each containing between 1 and 32 species (and between 1 and 3 plant functioning groups, namely grasses, legumes and other forbs). In total this produces 1934 data points per year, with each data point reporting the biomass of a species in an individual plot. Each monoculture or species mixture was replicated in two identical plots (with a few exceptions: five plant assemblages were replicated 4 times, see (Spehn et al. 2005)).

To standardise fluctuations relative to changes in mean productivity in time and space we quantified variability as the coefficient of variation (CV) where CV is the ratio of the standard deviation, σ, to the mean, μ, expressed as a percentage:

$CV = \sigma/\mu \times 100$

Spatial and temporal CVs were calculated for the biomass of individual species, functional groups and the experimental communities they composed. Temporal variability was quantified as the CV of aboveground biomass over the first three years of the BIODEPTH experiment (longer time series exist for some sites but show similar patterns as long as weeding is maintained; (Pfisterer et al. 2004). Spatial variability was quantified as the CV between the paired replicates of individual plant assemblages (particular species or mixtures of species, (see: Hector et al. 1999; Spehn et al. 2005) at each site. In the context of these measures, overyielding will have a stabilizing effect (reduced CV) when diversity increases the ratio of the mean relative to the standard deviation.

Analysis

The temporal and spatial CVs for the biomass of populations, functional groups and whole communities were analysed separately with mixed-effects models using the lme function from the nlme package implemented in R 2.8.0 (R Development Core Team 2007). Mixed-effects models use restricted maximum likelihood (REML) to estimate regression intercepts and slopes or treatment means (generally: 'intercepts') for fixed-effect explanatory variables (e.g. treatments) and to predict the variability (variance components) of slopes or intercepts for random effects (e.g. blocks). Following the BIODEPTH experimental design and our *a priori* hypotheses for this project, our analysis treats diversity (sown species richness) and organisational level (population, functional group or whole community) as fixed effects, reporting their point estimates with 95% confidence intervals. Sites were treated as random effects, as were years in the analysis of spatial CVs, allowing both the intercepts and slopes of the regression slopes versus diversity to vary with location and year as required. Species compositions were also treated as a random effect (nested within sites). The fixed-effect component of our models therefore examined the effects of diversity, level and their interaction. For the random-effect component of our models we followed a model building strategy (Pinheiro and Bates 2000) that uses likelihood ratio tests of models with and without a given random effect to determine which show significant levels of variation and are required in the model. The likelihood ratio test is based on the change in deviance (sums of squares) due to the removal of the random effect that is omitted from the reduced model. The change in deviance approximately follows a χ^2 distribution with the appropriate degrees of freedom and the test

tends to be conservative (Pinheiro and Bates 2000). Variance components for the random effects are reported as standard deviations (that is the square root of the variance component) on the same scale as the original measurements. To calculate the evenness of experimental and reference plots we used the reciprocal Simpson's index divided by the number of species (Magurran 2003), using biomass of species per plot instead of numbers of individuals. All reported intervals are 95% confidence intervals unless otherwise stated.

Results

Temporal and spatial variability

Our analyses of temporal and spatial variability both showed that the effect of diversity differed depending on organisational level (that is the diversity-by-level interaction was highly significant in both cases) with the results for the temporal and spatial CVs agreeing in part but differing in some ways (Fig. 1 and Fig. 2).

In the analysis of temporal variability (CV), diversity had a significant negative, stabilising effect at the community level as hypothesized (slope versus $\log_2$ species richness with 95% CI = -5.2 (-9.3 – -1.2); Fig. 1 left). In contrast, the effect of diversity on the population-level CVs was positive and destabilizing (slope = 11.1 (7.5 – 14.6); Fig. 1 right) as predicted, with the slope at the functional group level lying between the two (slope = 1.4 (-2.3 – 5.2); Fig. 1 middle). There was significant variation around these average fixed-effect slopes from site to site (likelihood ratio test: χ^2_2 = 10.6, P = 0.005; standard deviation of the variation in slopes across sites = 4.0 (1.9 – 8.6)). There was also significant variation in the temporal CVs of different species compositions within diversity levels (χ^2_1 = 181.6, P<0.001; SD of the variation in means for different compositions = 15.8 (13.6 – 18.3)).

In the analysis of spatial variability of individual species populations, the effect of diversity was never negative, that is never stabilizing (Fig. 2 bottom row). Species richness mostly had a positive, destabilising effect on spatial variability at the functional group level (Fig. 2 middle row) and mostly had no effect on spatial variability at the community level (Fig. 2 top row). The effect of diversity on spatial variability varied significantly with site (likelihood ratio test of log (species richness) for the intercepts

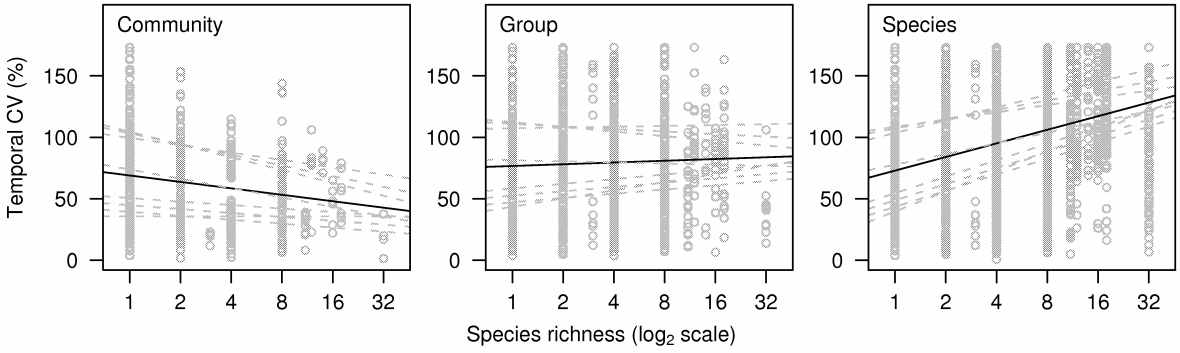

Figure 1. Temporal CVs as a function of diversity for species' populations, functional groups and whole communities. Solid black lines are the fixed-effect linear regression slopes for the overall response per level from the mixed-effects model while grey points and broken lines show the significant random effects variation for sites.

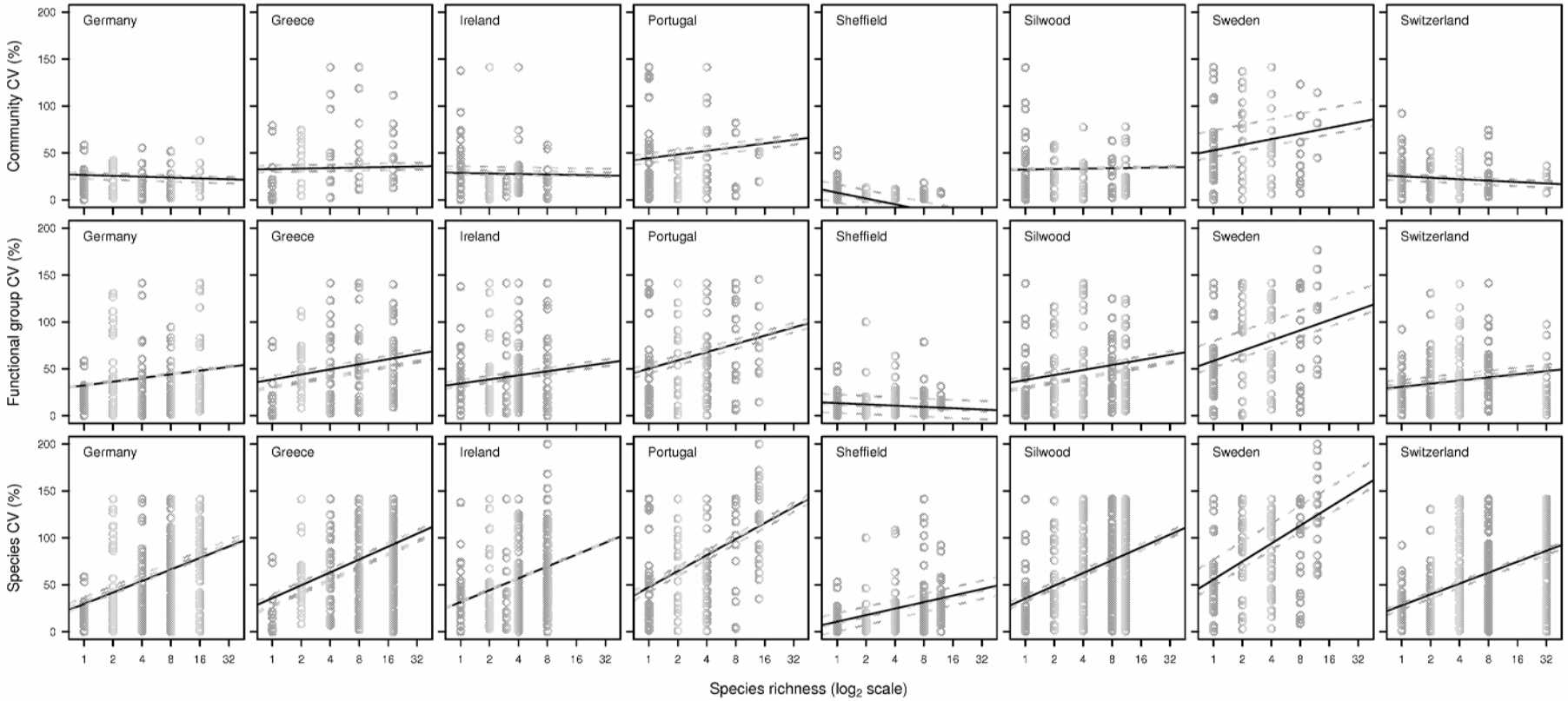

Figure 2. Spatial CVs as a function of diversity for species' populations (bottom row), functional groups (middle) and aggregate communities (top) at each site. Solid black lines are the fixed-effect linear regression slopes for the overall response per level from the mixed-effects model while grey points and broken lines show the significant random effects variation for different years and sites.

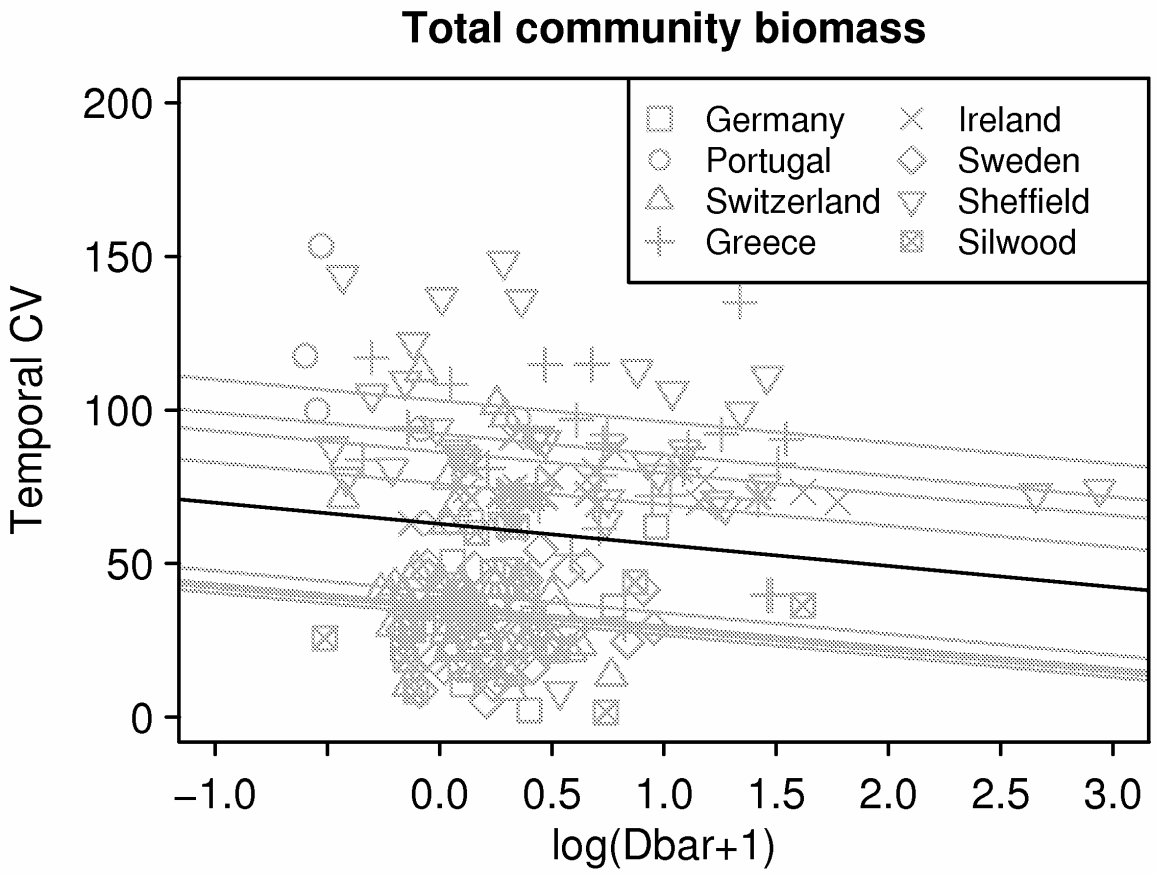

Figure 3. Temporal variability of aggregate community biomass (CV) as a function of overyielding ($\overline{D}$, averaged over three years, natural log-transformed after adding one). The solid black line is the significantly negative linear regression slope (the fixed effect for overyielding) from the mixed-effects model reported in the results and the grey symbols and lines in the background indicate the variability in the relationship across sites (the random intercepts for the overyielding relationship at different sites).

across sites: χ^2_2 =14.6, P<0.001 and for slopes across sites: χ^2_1 = 136.3, P<0.001; SD and SD of intercepts = 14.3 (8.0 – 25.6) of slopes = 3.8 (1.8 – 7.7); Fig. 2) with year affecting the magnitude of the effect; that is significant variation in the intercepts of the diversity relationships across years (likelihood ratio test of log (species richness) intercepts across years: χ^2_2 = 154.1, P<0.001; SD of intercepts = 19.7 (16.5 – 23.5)) but not in the slopes (likelihood ratio test of log (species richness) slopes across years: χ^2_1 = 4.6, P = 0.10; SD of slopes = 2.9 (0.7 – 12.3)). Once again, there was also substantial and significant variability between the different species compositions within sites (χ^2_1 = 194.2, P<0.001; SD = 8.3 (5.6 – 12.2)).

Stability and overyielding

Analyses using the additive partitioning method have shown widespread overyielding underlying positive diversity effects on aboveground biomass production within this dataset (Loreau and Hector 2001; Spehn et al. 2005). To examine the relationship between overyielding and stability we analysed the temporal community biomass CVs (reported above) as a function of average overyielding over the same time period (we used a relative measure of overyielding in line with the relative measure of variability given by the CV).

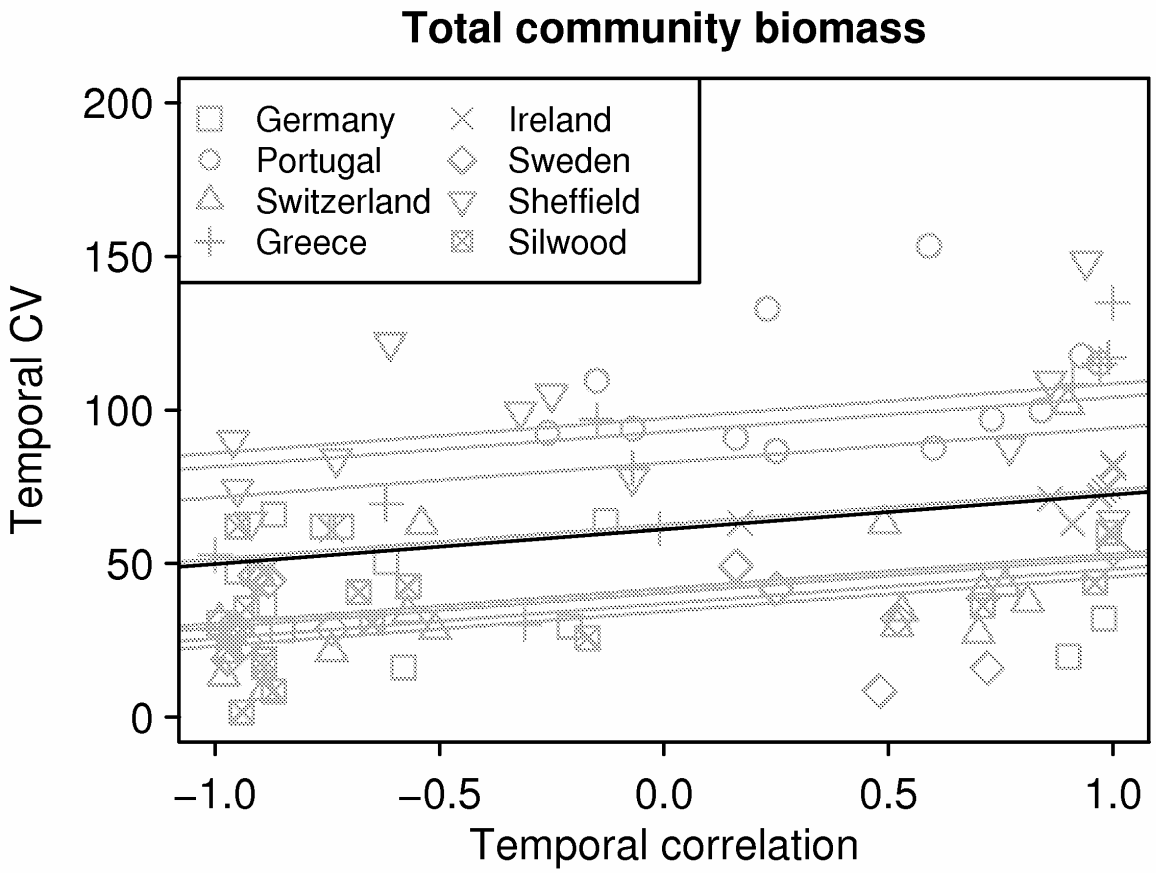

Figure 4. Temporal variability of total community biomass (CV) of two-species mixtures as a function of the temporal correlation between pairs of species in each mixture. Negative correlations (standardised negative covariances) are associated with greater temporal stability (lower temporal CV) as predicted by the insurance hypothesis and related theory. The solid black line is the significantly positive linear regression slope (the fixed effect for the temporal correlation) from the mixed-effects model reported in the results and the grey symbols and lines in the background indicate the variability in the relationship across sites (the random intercepts for the relationship at different sites).

Specifically, we used Loreau's (1998) $\overline{D}$, which quantifies the deviation of mixtures yields from a null expectation which in our case is the average of the monoculture yields. Since the temporal CV is calculated over three years we took the mean $\overline{D}$ averaged over the same period. To get a good distribution of $\overline{D}$ values along the x-axis in the analysis we took the natural log after adding one to remove zeros. We found a significant negative, stabilizing relationship between temporal variability (temporal CV) and overyielding (slope with 95% CI = -6.9 (-13.2 – -0.5); Fig. 3). There was substantial and significant variation across sites (χ_1^2 = 85.0, P<0001; standard deviation of the intercepts for different sites = 29.9 (17.2 – 51.8)) but the variation in the slopes was not significant (χ_2^2 = 1.0, P = 0.6, SD = 4.4 (0.6 – 32.4)). Within sites there was also significant variation in the variability of different species compositions (χ_1^2 = 12.4, P = 0.004; SD of the means for different species compositions within sites and species richness levels = 12.1 (8.6 – 16.9)).

Stability and negative species interactions

Previous analyses of stability in biodiversity

experiments have used negative summed covariances to quantify competitive interactions between species (Lehman and Tilman 2000; Tilman 1999; Tilman et al. 1998). These analyses have failed to find that negative covariances are stronger in diverse communities and concluded that competitive interactions play little or no role in generating insurance or portfolio effects of diversity. However, Loreau & De Mazancourt (2008) have recently shown that negative covariances cannot reliably be used as indicators of compensatory competitive interactions in multispecies communities for the following reason. First, imagine a community of two species interacting over time under fluctuating conditions that sometimes favour one species and sometimes the other. Strong competition will lead to negative temporal covariance (or correlation) in the abundances of the two species; in years when one is competitively superior its abundance will be high and that of the other species low and vice versa. Now consider adding a third species: it can strongly negatively covary with one of the two species but not both since a negative correlation with one species inevitably leads to a positive correlation with the other. As more species are added to the community this effect becomes more widespread and the average correlation between species tends to zero despite strong competition within the community. Due to this effect, negative covariances cannot generally be used, as they currently are, to quantify the strength of negative species interactions within multispecies communities. New methods for identifying the contribution of species interactions to community stability are under development (Loreau & De Mazancourt 2008) but will require more detailed information and longer time series than we currently possess in our dataset.

For our dataset, we examined the correlations between the pairs of species in the two-species mixtures only (Fig. 4), since in this case the limitations detailed above do not apply. Our analysis confirmed that two-species communities with negative temporal correlations had lower temporal CVs (slope = 11.2 (5.6 – 16.9)), suggesting that negative species interactions had a stabilizing effect at this level of diversity. There was significant variation in the intercepts of this relationship at the different sites (χ_1^2 = 41.9, P<0.0001; SD of the regression intercepts for individual sites = 27.1 (15.6 – 47.1)), but not in their slopes (χ_1^2 = 3.1, P = 0.22, SD = 9.0 (3.1 – 25.8)), as well as substantial within-site variation between different species compositions χ_1^2 = 4.36, P = 0.036, SD = 9.0 (4.3 – 18.5)).

Discussion

In our analysis, the most consistent result is the destabilizing effect of diversity on the variability of individual species; an effect seen for both temporal and spatial CVs at all sites. Our results also support the idea that diversity can have the opposite, stabilizing effect at the community level, but we only found this effect for temporal and not spatial variability. However, it is hard to know how effectively we can compare temporal and spatial variability with our data since levels of external temporal variation (e.g. weather) were natural while spatial heterogeneity may be unrealistically low due to the deliberate efforts to reduce it during site preparation (through ploughing, soil sterilization treatments *etc.*). This can be most clearly seen in Sheffield where the experiment was established on a custom-built site with a sand substrate. The low spatial CVs in Sheffield relative to the other sites presumably results from low levels of spatial heterogeneity in the homogenous sand substrate.

Our results reveal marked differences in the effects of diversity on the variability of individual species, functional groups and on the productivity of the aggregate community. This contrast between stabilising and destabilising effects of diversity depending on the organisational level (species, functional

group and community) provides experimental agreement with the results of an earlier analysis of long-term observational field data from Inner Mongolia grasslands (Bai et al. 2004). In our analysis, the effects of diversity on the variability of individual functional groups lay midway between its stabilizing effects on aggregate communities and destabilizing effects on individual species. This result shows that the averaging effect of asynchronous fluctuations of individual functional groups is weaker than that of individual species, or to put it another way: there is substantial asynchrony in the fluctuations of species within functional groups in addition to the asynchrony of the groups themselves. Our results show that the stabilizing insurance effects of diversity cannot be provided by functional groups alone as defined in our study. However, it is important to remember that our groups were intended as functional effects groups (that is species expected to have similar effects on ecosystem functioning). An alternative approach that may be more appropriate to the study of stability would be the use of functional response groups (species expected to respond in a similar way to environmental perturbation). Nevertheless, species richness within functional groups may play a stronger role for the stability biomass production over time (this paper) than for biomass production within a given growing season (Hector et al. 1999; Spehn et al. 2005).

Our analysis of the stabilizing effect of diversity at the community level demonstrated significant but relatively weak effects of two proposed mechanisms. Although there was substantial variability between- and within-sites we found stabilizing effects of both overyielding and asynchronous species fluctuations on the temporal variability of different species. For the reasons explained above our analysis of the correlations in species interactions was restricted to two-species mixtures only and deeper investigation of these effects will require more detailed datasets and new analytical methods.

Theory on the effects of the population dynamics of individual species on stability usually gives all species equal biomass as a simplifying assumption (but see Schwartz et al. 2000). However, in nature, the potential stabilizing effects of species is strongly influenced by their abundance since species that remain at relatively low abundance are limited in the contribution they can make to

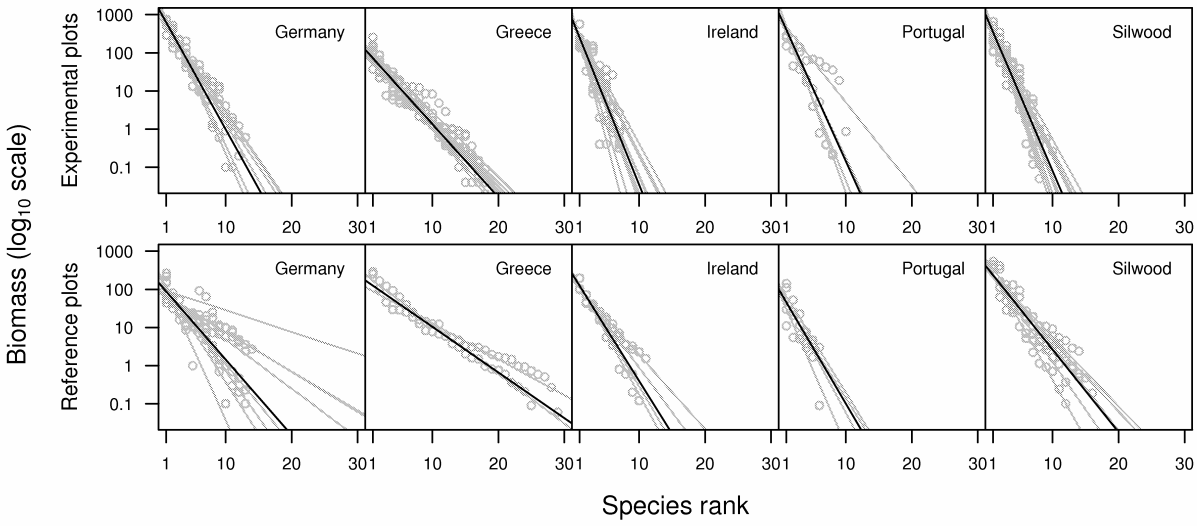

Figure 5. Comparison of rank-abundance curves for experimental and reference plots.

aggregate community biomass (Petchey et al. 2002). Because they usually sow or plant all species at equal initial density the experimental communities used in plant biodiversity experiments are often presumed to be unusually even and without the long tail of species at low relative abundances that are typical of natural communities. When this is the case, biodiversity experiments might over-estimate the potential insurance effects of diversity. For example, Polley et al. (2007) found that diversity had no stabilizing effect on the aboveground biomass production of remnant and restored prairie communities because temporal variability of production was driven by that of the dominant species. For five of our experiments we compared relative abundance distributions of the diverse experimental communities to matched "reference" plots in neighbouring natural grasslands (see Hector et al. 2007). It is hard to make a direct comparison since the experimental and reference plots differ somewhat in both their diversity and productivity but in general our experimental communities do not appear to be unnaturally even: they show a similar exponential decline in biomass when species are ranked from most to least abundant (Fig. 5). Unusually even distributions of abundance in our experimental communities therefore do not seem to be of particular concern. To formally analyse the abundance distributions of the most diverse experimental communities and compare them with those of the reference plots while controlling for differences in species richness we used Simpson's evenness index (that is reciprocal Simpson's diversity index divided by the number of species – see methods). A mixed-effects analysis containing site as a random effect showed no significant difference in evenness between the most diverse experimental communities and the natural reference plots (difference = -0.028; 95% CI= -0.116 – 0.060). While our experimental communities started with unrealistically even relative abundance distributions, patterns of dominance rapidly developed which were indistinguishable from those of the natural grasslands.

In summary, our results consistently support the prediction that diversity has a destabilising effect on the fluctuations of individual populations. This destabilising effect of diversity largely disappears at the resolution of plant functional groups and diversity had a stabilising effect on the productivity of aggregate communities of species through a combination of asynchronous fluctuations (species interactions and their differential responses to the environment) and overyielding. Our results confirm the predictions of the insurance hypothesis and, together with earlier studies, suggest that stabilising effects of diversity on ecosystem productivity may be relatively widespread in plant communities.

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